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Cu (II) Catalyzed Gateways In The Synthesis of Acridine Derivatives and Their Biological Evaluation as Anti-Cancer Drugs

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By

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Dedicated to my Parents

Mr. Siva Rama Krishnaiah Komati **and** ***Mrs. Ramadevi Komati***

And my beloved wife ***Mrs. Sushma Uppalapati***

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ABBREVIATIONS

DMF	*N,N*-Dimethyl formamide
DMSO	Dimethylsulfoxide
DCM	Dichloromethane
DCC	*N,N'*-Dicyclohexylcarbodiimide
DMAP	4-Dimethylaminopyridine
EtOAc	Ethyl Acetate
PTSA	*P*-Toluenesulfonic acid
NAM	N-Acridyl Maleimide
NAS	N-Acridyl Succinimide
NAP	N-Acridyl Phthalimide
A	Adenine
T	Thymine
G	Guanine
C	Cytosine
RT	Room Temperature
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
NMR	Nuclear Magnetic Resonance
h	hour
TLC	Thin Layer Chromatography

ABSTRACT

Telomeres are nucleoprotein complexes found at the ends of linear eukaryotic chromosomes. Telomeres consist of a short sequence of repetitive double stranded DNA, TTAGGG repeats in humans (and all mammals), and a complex of 6 proteins, termed the shelterin complex. The length of the telomeres varies greatly between species, from approximately 300 base pairs in yeast to many 10-15 kilo bases in humans, because of the end replication problem this length get shorten with each cell division and ultimately leads to cell death. However the immortal eukaryotic cells and some transformed human cells over come this incomplete end replication problem with the use of enzyme called Telomerase. Telomerase is a ribonucleoprotein enzyme that adds a specific DNA sequence repeats (TTAGGG) to the 3′ end of DNA strands in the telomere regions. However from the telomerase activity studies, it was concluded that telomerase is active in almost 90% of human cancers but not in normal somatic tissues. Finally, the low or transient expression of telomerase in normal tissues, including normal stem cells, and the generally longer telomeres in normal cells versus tumor cells provide a degree of tumor specificity to telomerase-based drugs and reduce the probability of toxicity to normal tissue. All of these factors suggest that cancer drugs based on telomerase might have a broad therapeutic window.

This dissertation focusing on the synthesis of acridine derivatives that have the capability to inhibit the enzyme telomerase. Several N-acridyl maleimide (NAM), N-acridyl succinimide (NAS) and N-acridyl phthalimide (NAP) derivatives have been synthesized and evaluated for their anti cancer activity against various cancer cell lines. While synthesizing acridine derivatives it was required to form the C-N bonds at various stages. Developed a copper-nicotinic acid

complex, which catalyzes the coupling of aryl halides with N-formyl amines and cyclic imides to form C-N bond. Explored Cu (II) catalyzed formation of C-N bond by coupling aryl halides with various N-nucleophiles such as formamide, *N,N*-dimethyl formamide, N-formyl amines and various cyclic imides.

Key Words: Telomeres, Telomerase, Eukaryotes, Shelterin Complex, End replication problem, Acridine, Anti cancer activity, Copper-Nicotinic acid complex, C-N bond, Aryl halides, N-formyl amines, Formamide, *N,N*-dimethyl formamide, Cyclic imides.

Chapter 1: Introduction

1.1Cancer:

Cancer medically known, as a malignant neoplasm is not a one disease but a large group of almost 100 diseases, which are a leading cause of death worldwide, accounting for 8.2 million deaths in 2012 [1]. However they all possess the same common property of abnormal cell growth.

Cancer cells majorly differ from somatic cells (normal cells) in 4-different ways [2],

1) Uncontrolled Proliferation: Proliferation occurs in normal cells as well as in cancer cells. But normal cells stop dividing after certain size has been reached, but not the cancer cells. This uncontrolled proliferation occurs in cancer cells due to elongation of "telomere by over active telomerase".
2) Loss of Contact Inhibition: Normal cells stop moving and growing when they come in direct contact with other cells called as "contact inhibition." cancer cells seem to have lost this.
3) Lack of Adhesion Requirement For Growth: Normal cells are adhere to one another and stay home, which is violated by cancer cells.
4) Inability to Differentiate Fully: A well-differentiated cell is "mature" and takes on a cellular function. It also looses its ability to divide, which is generally exhibited by normal cells.

Despite of this seriousness in disease, so far we don't have a perfect cure for cancer. Currently the most commonly used treatments to cure cancer are including surgery, radiation therapy and chemotherapy and several others [3]. All of theses have beneficial effects along with side effects.

These therapies used either alone or in combination with other therapies depending on the type of cancer.

Surgery: Surgery is the primary treatment for many types of cancer by which they remove the tumor and surrounding tissue by an operation. The side effects of surgery depend on the type of surgery and the overall health of the person before surgery. A common side effect is pain.

Radiation Therapy: In this treatment they use high energy X-rays or some other particles to kill cancer cells. There were several kinds of radiation therapies including external-beam radiation, internal radiation therapy or brachy therapy, and proton therapy among all external beam radiation is the common one.

Side effects of radiation therapy include fatigue, mild skin reactions, upset stomach and loose bowel movements, internal radiation therapy may cause bleeding, infection or irritation, all these are temporary and go away with time. It also associated with long-term side effects may include the risk of a second cancer, infertility heart problems, gastro intestinal problems, lung fibrosis, neurologic problems, thyroid problems or osteoporosis.

Targeted Therapy: Targeted therapy is a treatment that targets the cancer's specific genes, proteins, or the tissue environment that contributes to cancer growth and survival. It also blocks the growth and spread of cancer cells while limiting damage to normal cells. This treatment associated with side effects involving the skin, hair, nails and other areas of the body.

Immunotherapy: This is also called as biologic therapy, which designed to boost the body’s natural defenses to fight the cancer. It uses the material made either by the body or in a laboratory to bolster, target or restore the immune function. Side effects include flu like symptoms, such as chills, nausea and fever.

However, chemotherapy is the general treatment for all type of cancers, and in today's market more than 100 drugs are available to cure the cancer but most of them are cytotoxic because of lack of selectivity. However, the discovery of telomeres and telomerase [4] intrigue the scientists in developing the new class of anti cancer drugs that selectively targets the cancer cells.

1.2 Telomeres:

Telomeres are nucleoprotein complexes found at the ends of linear eukaryotic chromosomes (Figure 1.1) [5]. Most prokaryotes, lacking this linear arrangement, do not have telomeres. Telomeres consist of a short sequence of repetitive double stranded DNA, TTAGGG repeats in humans (and all mammals), and a complex of 6 proteins, termed the shelterin complex. These repetitive DNA sequences (TTAGGG) span approximately 10 to 15 kilobase pairs in humans at birth, and this length decreases with age [6].

Telomeres compensate for incomplete semi-conservative DNA replication at chromosomal ends. The protection against homologues recombination (HR) and non-homologues end joining (NHEJ) constitutes the essential " capping " role of telomeres that distinguishes them from DNA double strand breaks (DSBs) [7].

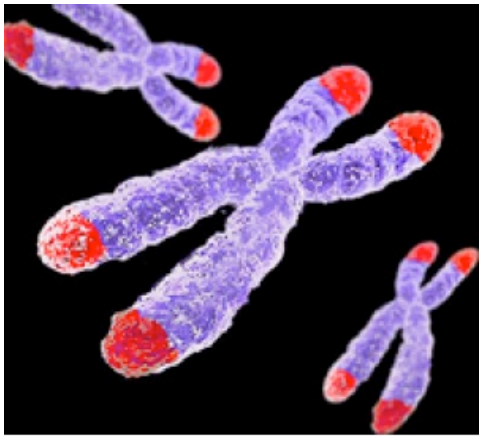


Figure 1.1 Purple color chromosomes capped with red color telomeres

1.2.1 Telomeres length with number of cell divisions:

The length of the telomere varies greatly between species, from approximately 300 base pairs in yeast to many 10-15 kilo bases in humans. However the length of the telomere get shorten with each cell division because of end replication problem (Figure 1.2) that is exhibited during DNA replication in eukaryotes only. It was estimated to be telomeres loose 50-100 base pairs at each cell division [8].

The DNA replication does not begin at either end of the DNA strand, but starts in the center, and considering that all known DNA polymerases move in the 5' to 3' direction, one finds a leading and a lagging strand on the DNA molecule being replicated. On the leading strand, DNA polymerase can make a complementary DNA strand without any difficult because it goes from 5' to 3'. However the other so called lagging strand is replicated discontinuously. In fact DNA polymerase III adds a multitude of small segments of nucleotides named Okazaki fragments, primed by short stretches of RNA primers. In the next step another type of enzyme, DNA polymerase I removes RNA primers, replacing the ribonucleotides with deoxyribonucleotides by extending the strand from the adjacent Okazaki fragments. The primers can be replaced everywhere except at the extreme 5' end, which makes this new strand slightly shorter than the parallel one [9].

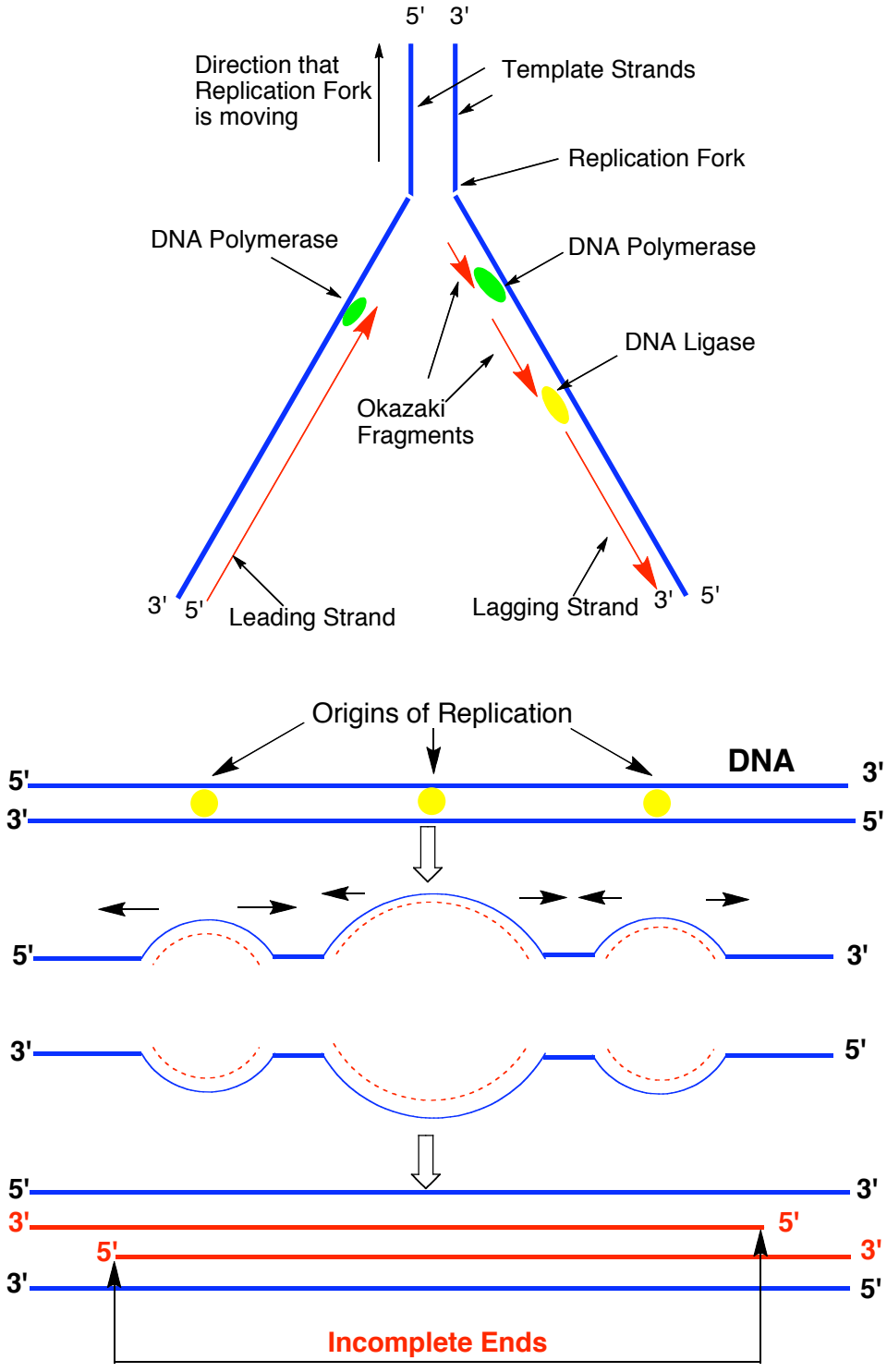


Figure 1.2 DNA end replication proble

It was predicted that after 20-40 generations of cell doubling, the telomere length shorten completely and prevent cells from further duplicating, causing cellular senescence and irreversible cell death. However the immortal eukaryotic cells and some transformed human cells over come this incomplete end replication problem with the use of enzyme called Telomerase (Figure 1.3) [10].

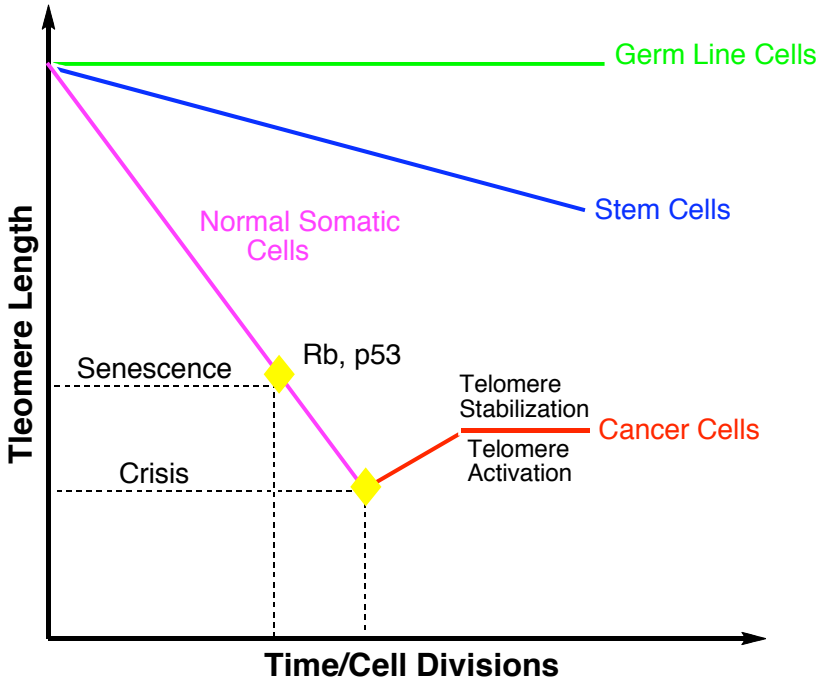


Figure 1.3 Telomere lengths with number of cell divisions for various cell types

1.3 Telomerase:

Telomerase is a ribonucleoprotein enzyme that adds a specific DNA sequence repeats (TTAGGG) to the 3′ end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes. Carol W. Greider and Elizabeth Blackburn discovered Telomerase in 1984 in the ciliate *Tetrahymena* [11].

Human telomerase consists of two molecules each of human telomerase reverse transcriptase (TERT), telomerase RNA (TR or TERC), and dyskerin (DKC1).

1.3.1 Elongation telomeres by telomerase:

The telomerase reverse transcriptase enzyme (TERT) uses TERC to add a six nucleotide repeating sequence, 5′- TTAGGG to the 3′ strands of the chromosomes. The figure shows the mechanism of elongation (Figure 1.4).

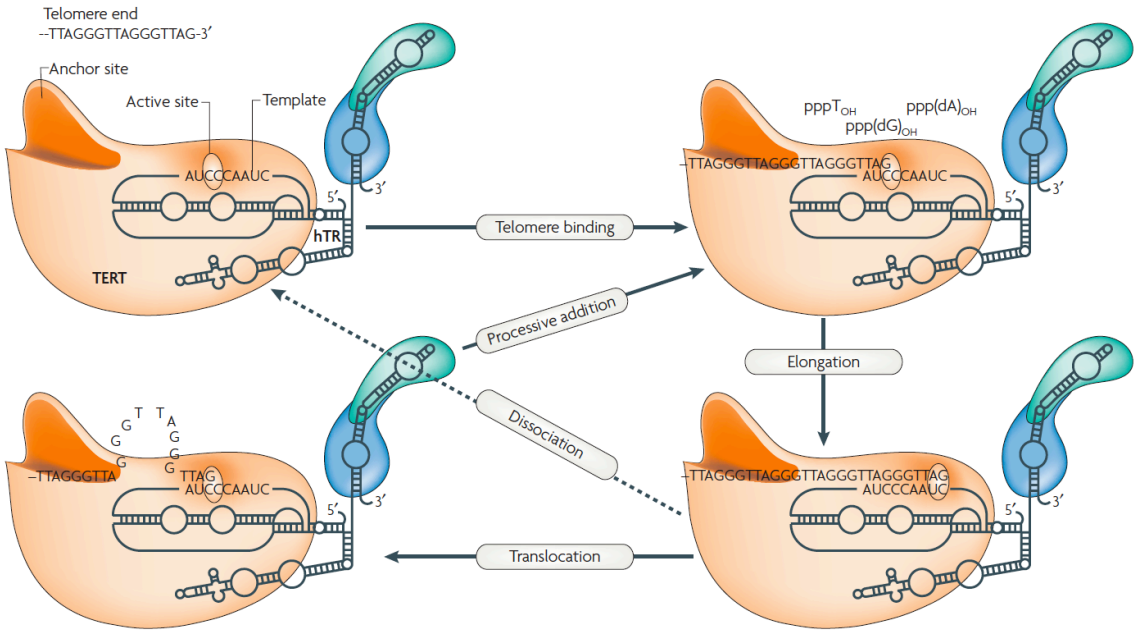


Figure 1.4 Mechanism of the elongation of the telomeres by telomerase

The elongation process takes place via the following steps:[12]

1) Telomere binding, in which the 3′ end of the G-rich telomeric strand aligns opposite the complementary (C- rich) template region.
2) Elongation, in which six nucleotides (GCTTAG, directed by the complementary template sequence0 are sequentially, added to the telomere.
3) Translocation, in which the extended telomere-template heteroduplex is interrupted and shifted back by six nucleotide, positioning the enzyme for another round of elongation, that is processive addition of GGTTAG.

However the whole process can be interrupted when the extended telomere dislocates from telomerase.

1.3.2 Activity of Telomerase in normal and cancer cells:

Telomerase is a ribonucleoprotein complex that extends and maintains the telomeres, and activation of this enzyme is therefore required for cells to overcome replicative senescence and obtain the ability to divide without limits. This concept was supported by findings that telomerase activity is observed in the vast majority of cancers or cancer cell lines but not in most normal tissues [13]. However, along with the cancer cells, some types of normal cells like hematopoietic progenitor cells, intestinal crypt cells, endometrial cells and basal layer cells of skin and cervical keratinocytes express telomerase activity [14].

Kim et al, conducted a detailed experiments on the activity of telomerase in both normal and cancer cells [15]. They conducted a survey on the telomerase activity and this survey included a total of 100 immortal cell lines and 22 normal somatic cell cultures from 18 different tissues. Of

the 100 immortal cell lines, 94 were tumor derived lines and 6 were cell lines transformed with viral oncoproteins.

Table 1.1 Telomerase activity in normal and immortal cells.

Tissue of Origin	Cell Type	Telomerase Activity (no. Positive/ no. Tested)
Skin	Tumor	8/8
Skin	Normal	0/5
Connective	Tumor	1/1
Joint	Normal	0/1
Adipose	Tumor	1/1
Breast	Tumor	22/22
Breast	Normal	0/8
Lung	Tumor	18/18
Lung	Transformed	2/3
Lung	Normal	0/3
Stomach	Tumor	1/1
Pancreas	Tumor	3/3
Ovary	Tumor	5/5
Cervix	Tumor	3/3
Cervix	Normal	0/1
Uterus	Tumor	0/1
Kidney	Tumor	8/8

Kidney	Transformed	1/1
Bladder	Tumor	3/3
Bladder	Normal	0/1
Colon	Tumor	7/7
Prostate	Tumor	2/2
Prostate	Transformed	0/1
Prostate	Normal	0/2
CNS	Tumor	3/3
Retina	Transformed	1/1
Blood	Tumor	9/9

From the table 1.1, it was clear that all of the 94 tumor lines are telomerase active and among the 6-transformed tumor lines 2 lines have been found to be telomerase inactive and all 22-cell lines derived from normal somatic cells are telomerase inactive.

The TRAP assay on wide range of normal and tumor tissues concluded that, 90 of 101 malignant tumor samples expressed high telomerase activity.

Table 1.2 Telomerase activity in human tumors and tissues

Tissue Type	**Telomerase Activity (no. Positive/no. Tested)**
Fetal testis	2/2
Adult testis	1/1
Fetal ovary	2/2

Ovarian follicle	1/1
Hepatocellular carcinoma	1/1
Colon cancer	8/8
Adjacent colonic tissue	0/7
Colonic tubular adenoma	0/1
Colonic polyp	0/1
Squamous cell carcinoma (head and neck)	14/16
Adjacent tissue	6/16
Normal breast tissue (from non cancer patients)	0/8
Prostate cancer	2/2
Prostatic intraepithelial neoplasia type 3	3/5
Benign prostatic hyperplasia	1/10
Normal prostatic tissue	0/8
Neuroblastoma	5/5
Brain tumors	6/8
Lung small-cell carcinoma	4/4
Rhabdomyosarcoma	1/1
Leiomyosarcoma	3/3
Leiomyoma 9fibroids)	0/11
Normal myometrium	0/10
Acute lymphocytic leukemia	14/16
Chronic lymphocytic leukemia	2/2

Lymphoma (adult)	5/5
Wilms tumor	6/6
Adjacent kidney tissue	2/6
Breast cancer (ductal and lobular, node positive)	18/20
Breast cancer (axillary node negative)	1/4
Adjacent tissue	2/20

The structural studies of the telomerase enzyme complex have revealed the presence two major subunits contributing to enzyme activity: a structural RNA component (hTER) that contains a template region that binds the TTAGGG repeats in telomeres [16] and a catalytic subunit with reverse transcriptase activity (hTERT). While hTER is constitutively present in normal and cancer cells, expression of hTERT is almost exclusively limited to cancer cells [17]. This was full supported by the fact that, introduction of hTERT gene into telomerase-negative normal cells is sufficient to induce telomerase activity and to immortalize cells that can be propagated to telomere based replicative senescence [18].

Various research studies on transcriptional regulation of the hTERT promoter identified the factors including c-Myc and Sp1 critically regulate the promoter activity [19]. However, most factors are not stringently tumor-specific and are also expressed in some normal cells that lack telomerase activity. Thus, the tumor specific hTERT expression cannot be explained based on these transcription factors. Unknown 'repressors' that are expressed in normal cells but are absent in cancer cells may explain the specificity of hTERT in tumor cells. Unfortunately, such transcriptional repressors have not yet been identified [20]. The regulation of epigene by DNA

methylation and histone acetylation is also insufficient to explain the tumor specificity of hTERT expression. Despite of the extensive efforts by a number of groups, the mechanisms of tumor specific telomerase activation are not fully established.

However from all these telomerase activity studies, it was concluded that telomerase is active in almost 90% of human cancers but not in normal somatic tissues. Finally, the low or transient expression of telomerase in normal tissues, including normal stem cells, and the generally longer telomeres in normal cells versus tumor cells provide a degree of tumor specificity to telomerase-based drugs and reduce the probability of toxicity to normal tissue. All of these factors suggest that cancer drugs based on telomerase might have a broad therapeutic window [21].

1.3.3 Targeting Telomerase:

The key advantages of targeting telomerase in comparison with most other cancer targets are its relative universality, criticality and specificity for cancer cells, including the putative cancer stem cell [22]. The various approaches to kill the telomerase positive cancer cells had been shown in the Figure 1.5.

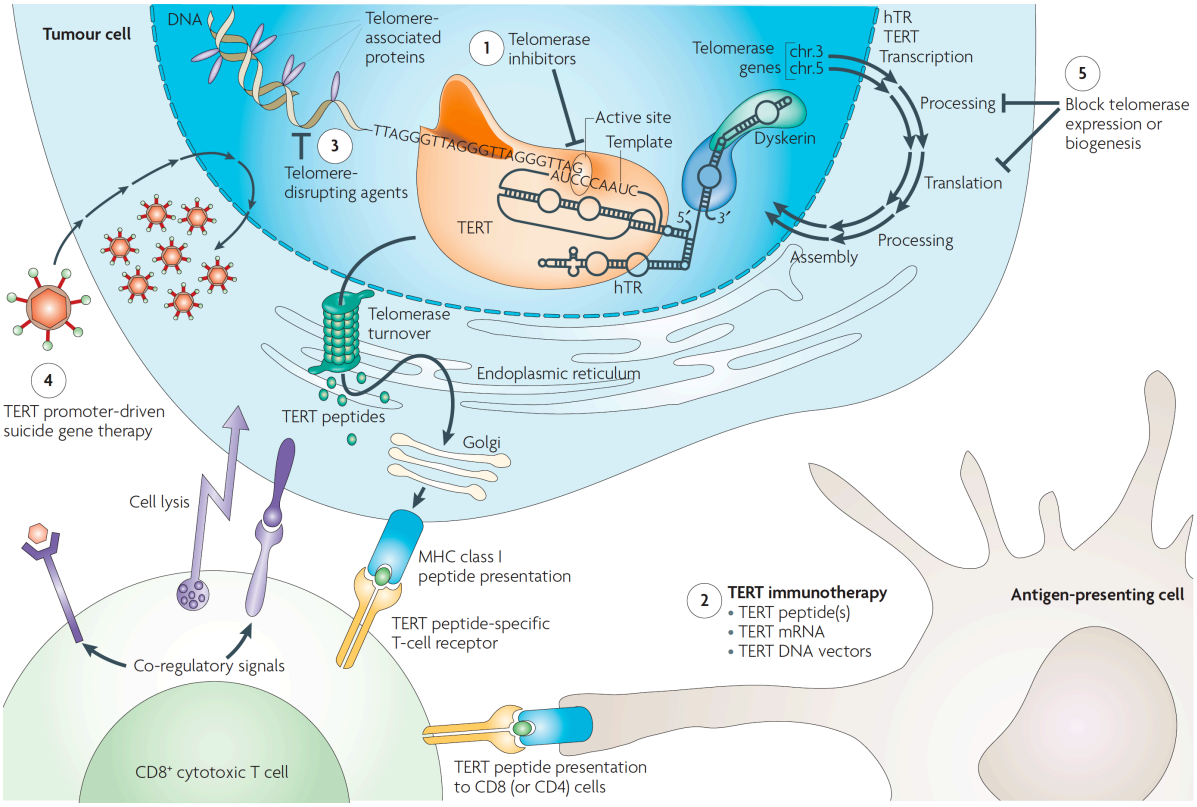


Figure 1.5 Available approaches to target the telomerase enzyme

The 5-approaches that are available to target the telomerase are [23]

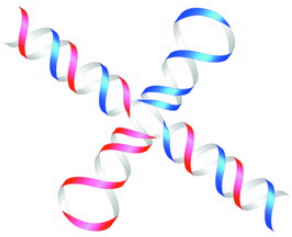
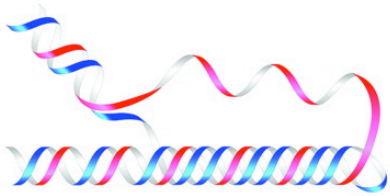
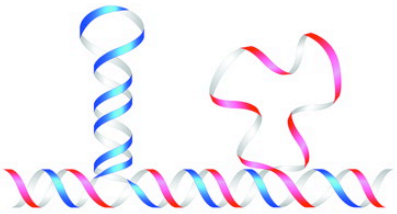
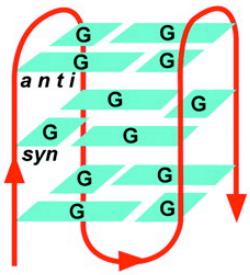
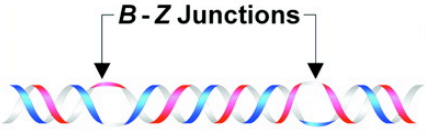
1) Telomerase is a unique enzyme, whose function is to synthesize telomeric DNA. So the most obvious approach is direct enzyme inhibition. Several strategies are possible, including the active site inhibitors that mimic telomeres or the nucleotide substrates, and allosteric inhibitors, which target structural features of telomerase reverse transcriptase (hTERT) or telomerase RNA component (hTR).
2) The second approach is the active immunotherapy. Telomerase immunotherapy products are designed to stimulate the patient's immune system to attack and kill telomerase-positive tumor cells that express TERT.

3) The third approach uses telomere-disrupting agents to alter the structure of the telomeres leading to inability of telomerase to access the telomere, or to a telomere length-independent damage signal causing immediate cell arrest or death.
4) The fourth approach is the suicide gene therapy, where the delivery of suicide gene in a viral vector and production of the toxic gene product in the cytoplasm which targets the promoter region of hTERT.
5) The fifth approach is blocking telomerase expression or biogenesis is based on the growing understanding of how the telomerase enzyme is made, from transcription to post translational modification, assembly and transport.

1.4 Telomeres G-quadruplex structure:

The right-handed double helical structure of B-form DNA has been known since long time [24]. However, it has become increasingly clear that DNA can adopt a variety of alternative conformations based on particular sequence motifs and interactions with various proteins. A number of non-B DNA structures have been discovered (approximately one new confirmation every 3 years for the past 35 years) and include the following: triplexes, left-handed DNA, bent DNA, cruciforms, nodule DNA, flexible and writhed DNA, G4 tetrad (G-Quadruplex or Tetraplexes), slipped structures, and sticky DNA (Table 1.3) [25]. All these structures were originally characterized *in vitro* by using biophysical techniques such as circular dichroism [26].

Table 1.3 Non B-DNA conformations involved in rearrangements

Name	Conformation	General Seq. Requirements	Sequence
Cruciform		Inverted Repeats	TCGGTACCGA AGCCATGGCT
Triplex		$(R \bullet Y)_n$ Mirror Repeats	AAGAGG GGAGAA TTCTCC CCTCTT
Slipped (Hairpin) Structure		Direct Repeats	TCGGTTCGGT AGCCAAGCCA
Tetraplex		Oligo $(G)_n$ Tracts	$AG_3(T_2AG_3)_3$ single strand
Left-handed *Z* - DNA		$(YR \bullet YR)_n$	CGCGTGCGTGTG GCGCACGCACAC

Human telomeric DNA contains thousands of tandem repeats of the G-rich $(TTAGGG)_n$ sequence. However, in molecular biology the guanine rich nucleic acid sequences have the tendency to form G-quadruplexes (G-Tetrads or G_4- DNA) structures (Figure 1.8), those are arises from the Hoogsten hydrogen bonding [27].

In contrast to Watson- Crick bonding (Figure 1.6) which involves N1 and N3 of the heterocyclic rings, Hoogsten bonding (Figure 1.7) involves N7, and occurs between this N7 and N3 on the corresponding nucleotide.

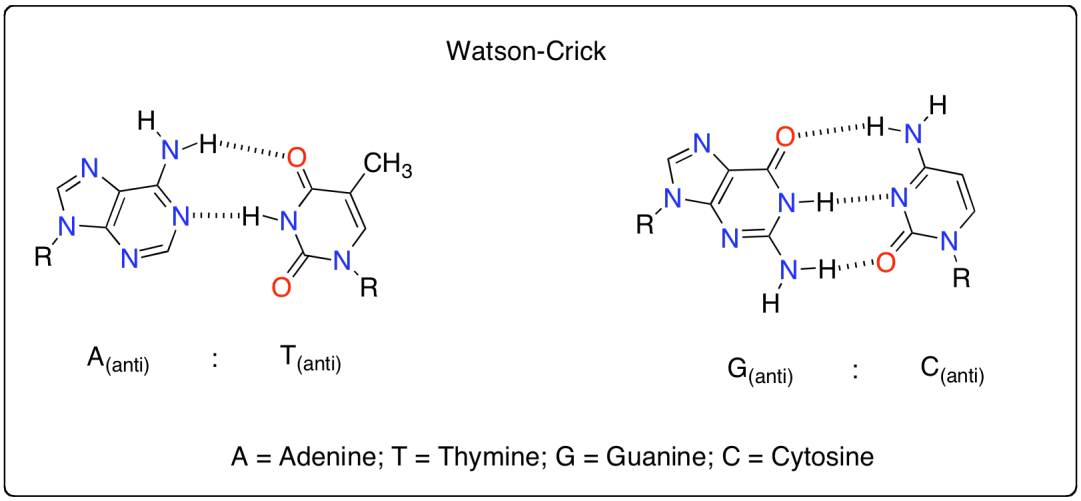


Figure 1.6 Watson-Crick H-bonding

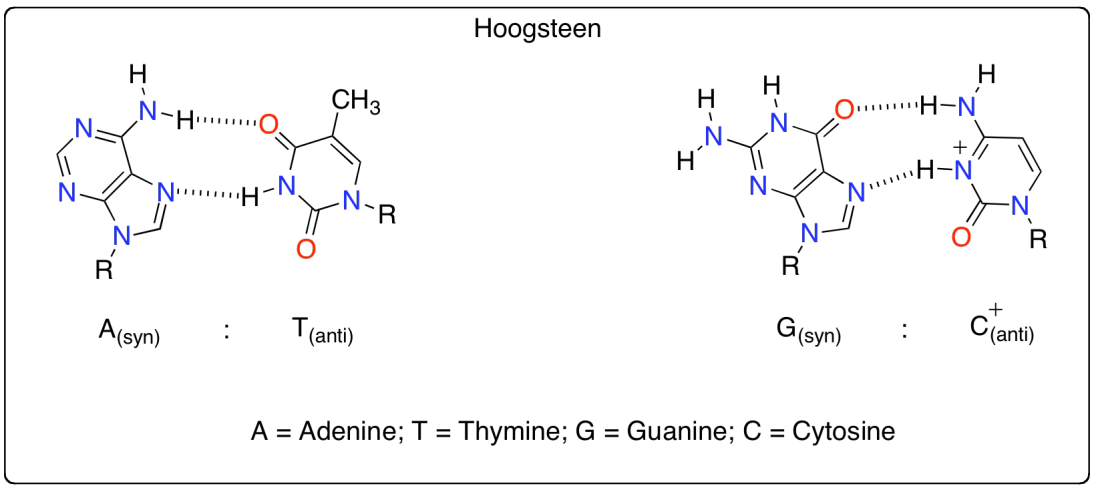


Figure 1.7 Hoogsteen H-bonding

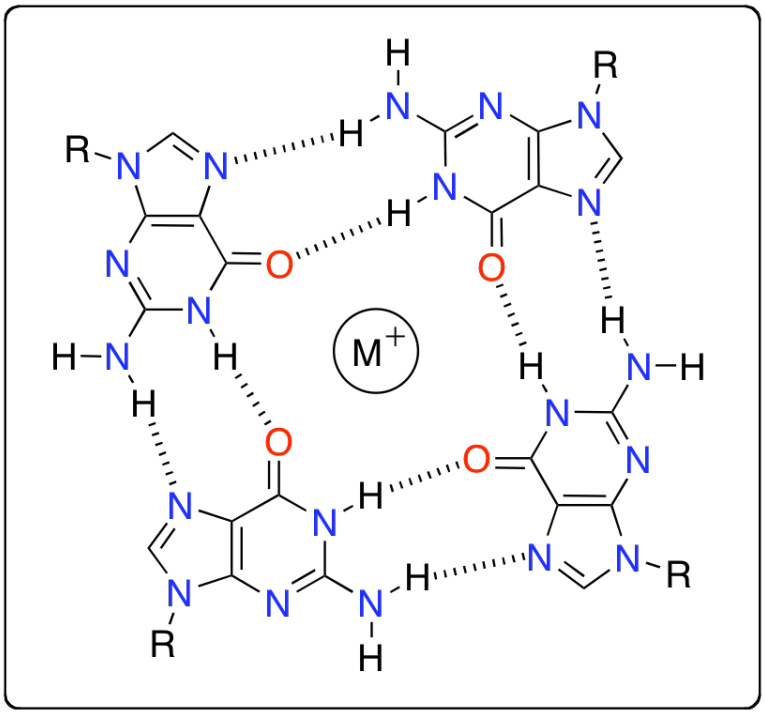


Figure 1.8 G-quartet formed by Hoogsteen H-bonding

1.4.1 Quadruplex Topology and Structure:

Quadruplexes can be formed from one, two or four separate strands of DNA (or RNA) and can display a wide variety of topologies, which are in part a consequence of various possible combinations of strand direction, as well as variations in loop size and sequence [28].

There are 3 different types of G-quadruplexes available.

(1) Unimolecular (Intramolecular): The sequences $G_m X_n G_m X_o G_m X_p G_m$, where m is the number of G residues in each short G-tract, which are usually directly involved in G-tetrad interactions. X_n , X_o , and X_p can be any combination of residues, including G, forming loops.

(2) Bimolecular: The association of two identical sequences $X_n G_m X_o G_m X_p$, where n and p may or may not be zero, forms Most of the bimolecular sequences reported to date.

(3) Tetramolecular: tetramolecular quadruplexes may be formed by four $X_n G_m X_o$ or $G_m X_n G_m$ strands associating together.

All these G-quadruplex structures exist in different topologies

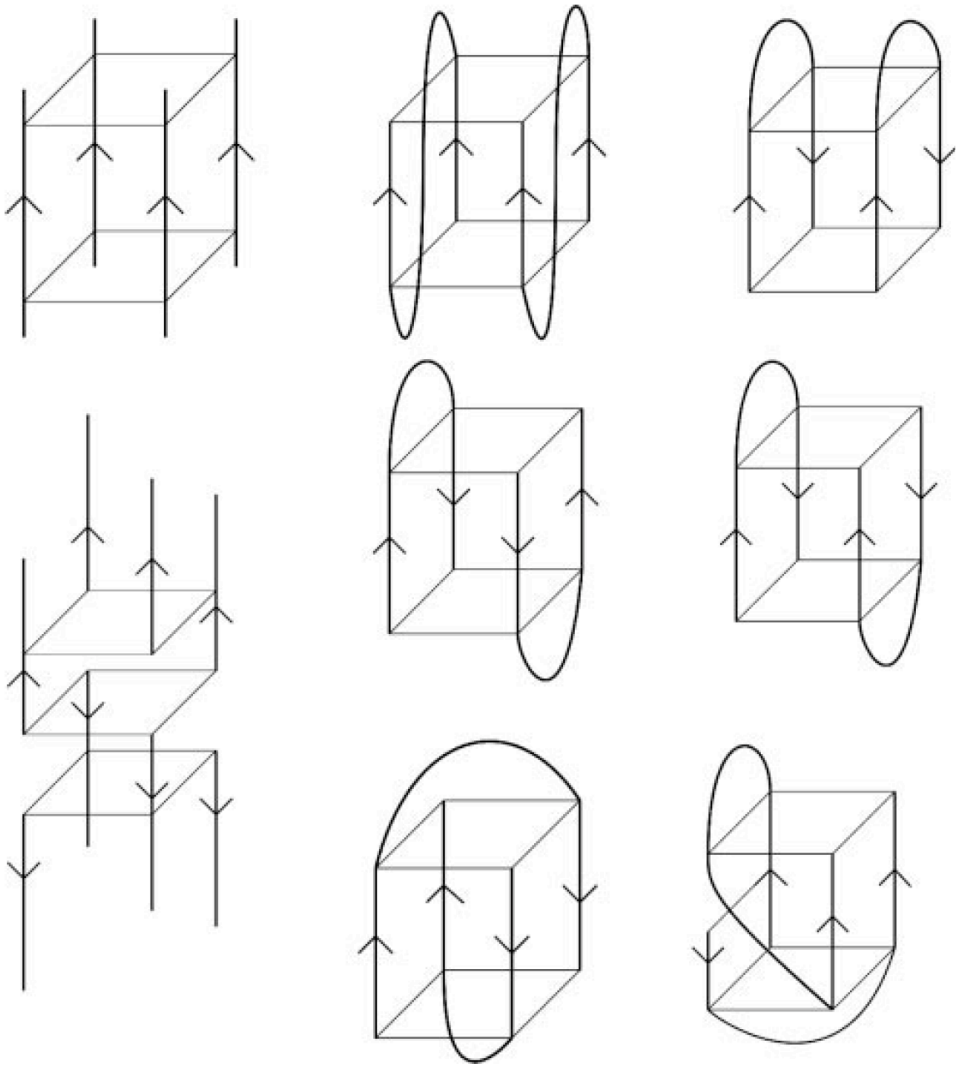


Figure 1.9 Some possible topologies for simple tetra molecular (on the left hand side) and bimolecular quadruplexes.

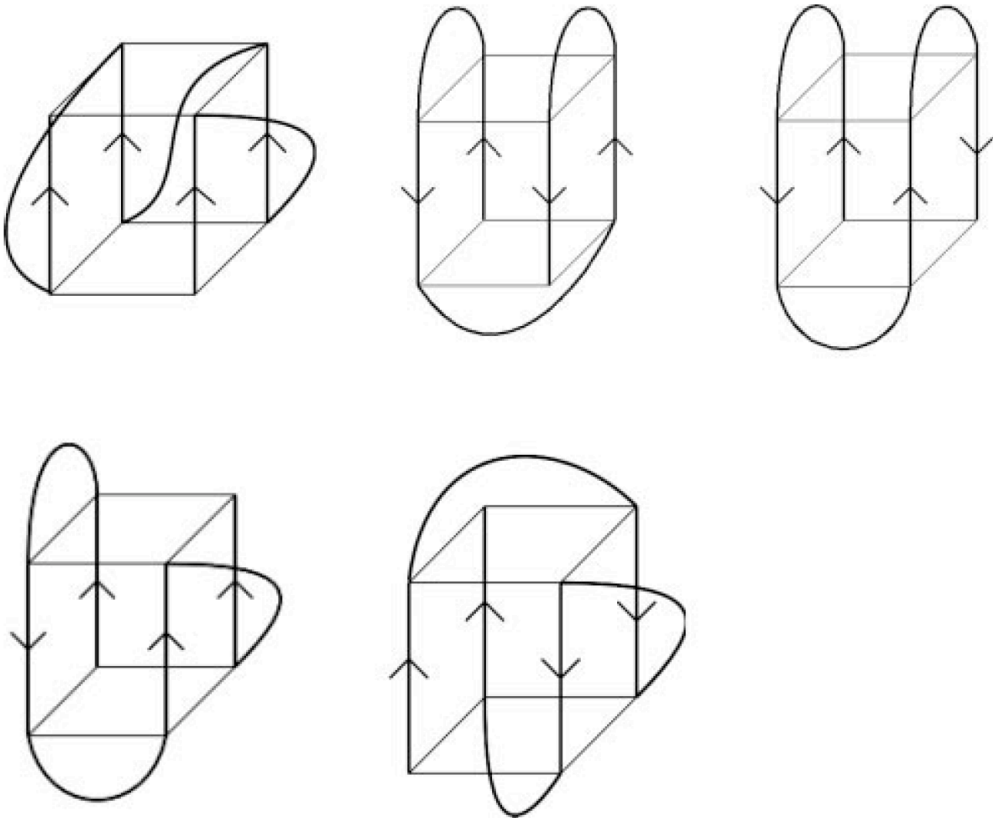


Figure 1.10 Some possible topologies for simple unimolecular quadruplex

Most of the vertebrate telomeric sequence d(TTAGGG) forms the unimolecular quadruplexes. There is a good evidence from a range of biophysical techniques, that the four-repeat quadruplex formed by the sequence $d(TTAGGG)_4$ (and variants on it, notably $d[AGGG(TTAGGG)_3]$), adopt differing topologies in Na^+ versus K^+ solution [29].

NMR analysis [30] of the structure formed in Na^+ conditions by the 22mer $d[AGGG(TTAGGG)_3]$ has shown that the structure has an anti-parallel fold with two lateral and one diagonal loops, each loop comprising the TTA triad sequence (Figure 1.11).

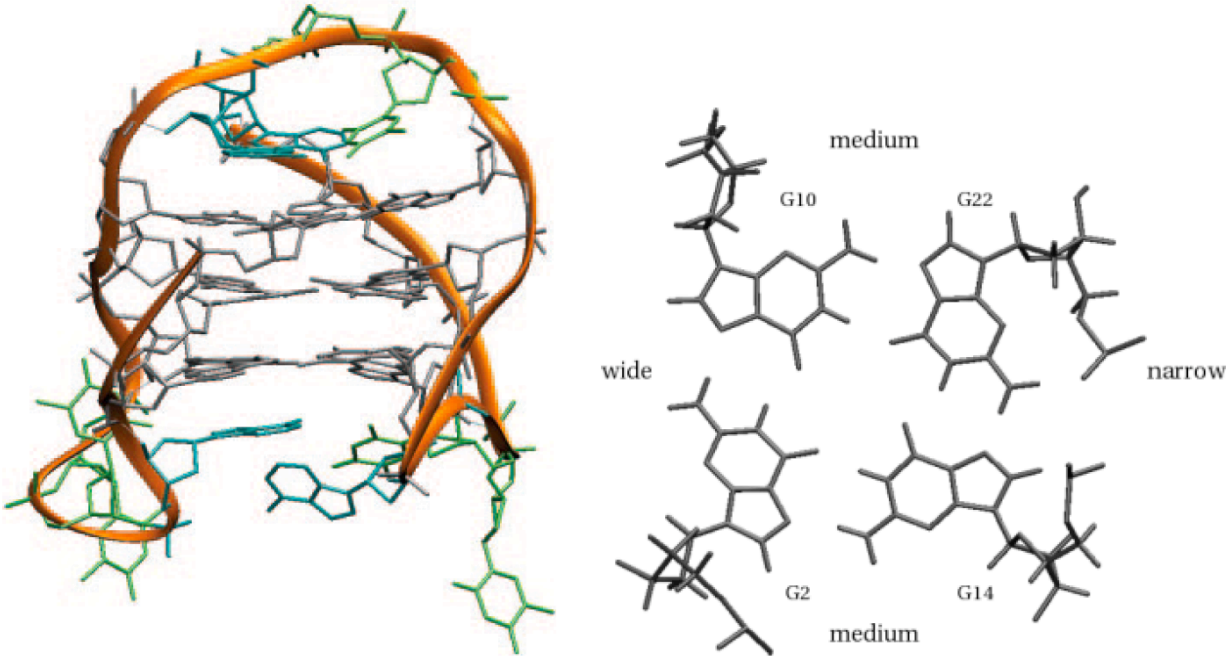


Figure 1.11 The deposited structure of the Na^+ form of human unimolecular telomeric quadruplex formed from the sequence d[AGGG(TTAGGG)$_3$].

The crystallographic analysis [31] of this sequence and the related 12mer (i.e two-repeat) sequence d(TAGGGTTAGGGT), in K^+ solution, showed that they form a unimolecular (Figure 1.12) and a bimolecular quadruplex, respectively in the crystal lattice.

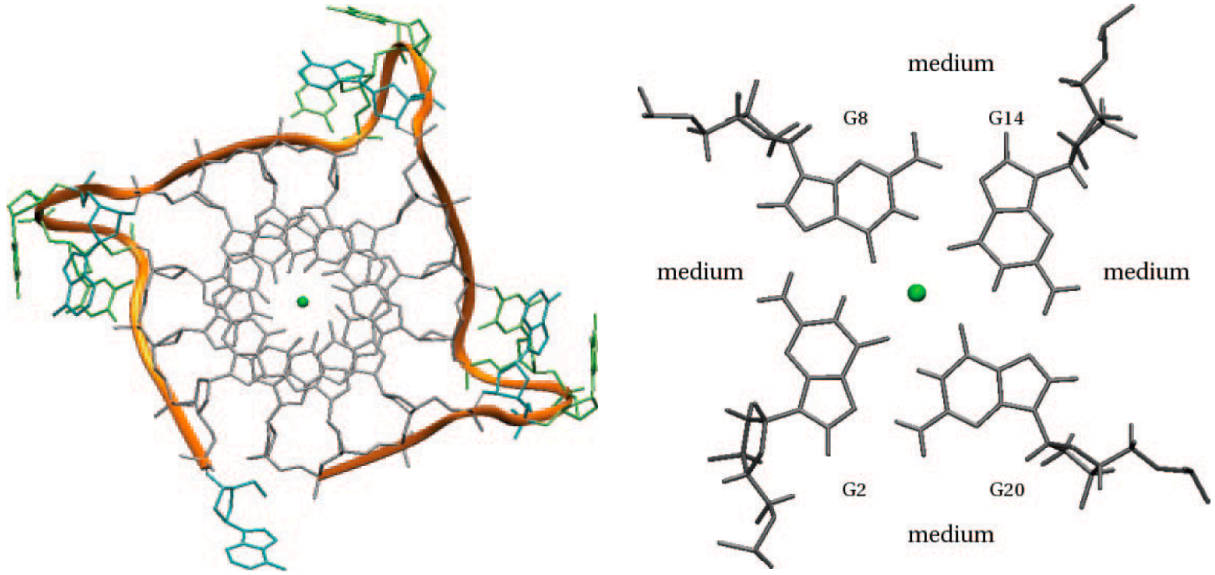


Figure 1.12 The K^+ form of human unimolecular telomeric quadruplex formed from the sequence d(TAGGGTTAGGGT).

Most importantly, the implication of G-quadruplex is evoked in several biological dysfunctions that selectively alter the integrity of cancer cells [32]. In particular, the formation of G-quadruplex DNA at the end of telomeres has been reported not only to impede the telomerase association and activity (due to the enzyme inability to bypass the folded form of its DNA-substrate) but also severely to increase the genomic instability by hampering normal recognition of telomere-associated proteins with their targets [33]. The regulatory potential of G-quadruplexes towards cancer cell growth is also strongly substantiated by their possible formation in the promoter regions of several human genes (such as the retinoblastoma susceptibility [34], insulin [35], muscle specific [36], vascular endothelial growth factor [37], hypoxia inducible factor 1α [38], fragile X mental retardation genes [39]) and oncogenes (such as c-myc [40], k-ras [41], bcl-2 [42], c-kit [43] or RET oncogenesis [44]). Consequently the possibility of building novel anti-cancer therapeutic strategies with G-quadruplex-DNA as the cornerstone is currently under investigation.

Therefore a general consensus is that G-quadruplex binders that stabilize the G-quadruplex structure could pave the way for the discovery of novel anti-cancer drugs.

1.5 Targeting G-quadruplex structure by using small molecules:

The first small molecule telomerase inhibitor based on G-quadruplex stabilizing was reported by SUN group in 1997 [45], since then different research groups from around the world developed various types of telomerase inhibitors that stabilizes the G-quadruplex structure of the telomeres.

To design the small molecules that specifically target the G-quadruplex structures it is important to consider the G-quadruplex and ligand interactions. Majority of the small molecule ligands that are reported possesses the binding mode of pi-pi stacking and electrostatic interactions, so usually the aromatics and charged ligands have been widely discussed. We also have the reports where the ligands bind with the quadruplex grooves, convex loops and negatively charged ion channels. However the small molecules that target the g-quadruplex have been divided into 3-main categories based on their structural characteristics and nature of binding.

1) Flat aromatic rings with arms or side chains.
2) Flat aromatic ring systems.
3) Macro cyclic ligands.

1.5.1 Flat aromatic rings with arms or side chains:

Most of the G-quadruplex ligands that are designed to date are flat aromatic compounds that targets the G-tetrad of the quadruplex, in order to anchor them onto the G-quadruplexes those flat aromatic ring systems are functionalized with arms or sidechains, these side chains are usually amines such as pyrrolidines, quinolines or (N,N-dimethyl)- ethylene diamines. These diamines are usually protanated at physiological pH conditions and engaged in electrostatic interaction

with the negatively charged DNA or phosphate backbone. Examples such as PIPER, TMPy et al comes under this category.

1.5.1(a) PIPER: perylene compounds are highly versatile molecules which attract a great interest owing to their applications in diverse fields of physical organic chemistry, such as dye lasers, light harvesting arrays, organic electronic devices and liquid crystalline dyes [46]. Further more perylene derivatives have been widely studied in medicinal chemistry because they can be considered potential antitumor drugs acting as telomerase inhibitors [47]. Perylene diimides, with their five condensed aromatic ring systems with suitable polar side chains can be considered potentially good G-quadruplex interacting compounds.

PIPER [48] ((*N,N*'-Bis[2-(1-piperidino)-ethyl]-3,4,9,10-perylenetetracarboxylic diimide) (Figure 1.13) the fused perylene ring system has a strong interaction with the G-quadruplex structure. According to the NMR studies by Hurley and co workers, the ligand molecule doesn't intercalate with in the G-quadruplex itself but rather stacks on the surface of the 3'- terminal G-tetrad (Figure 1.14). This binding mode can be classified as a " threading intercalation " with a fast structural transition between the two orthogonal drug orientations (Figure 1.15). From the results of gel shift experiments the authors also concluded that PIPER can dramatically accelerates the association of a DNA oligomer containing two tandem repeats of the human telomeric sequence (TTAGGG) into di- and tetrameric G-quadruplexes.

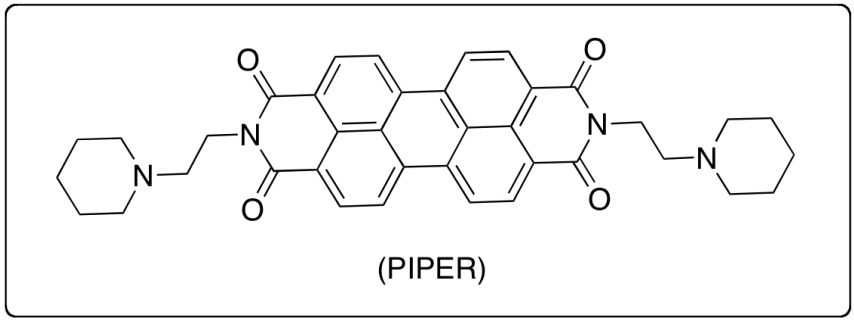


Figure 1.13 PIPER structure

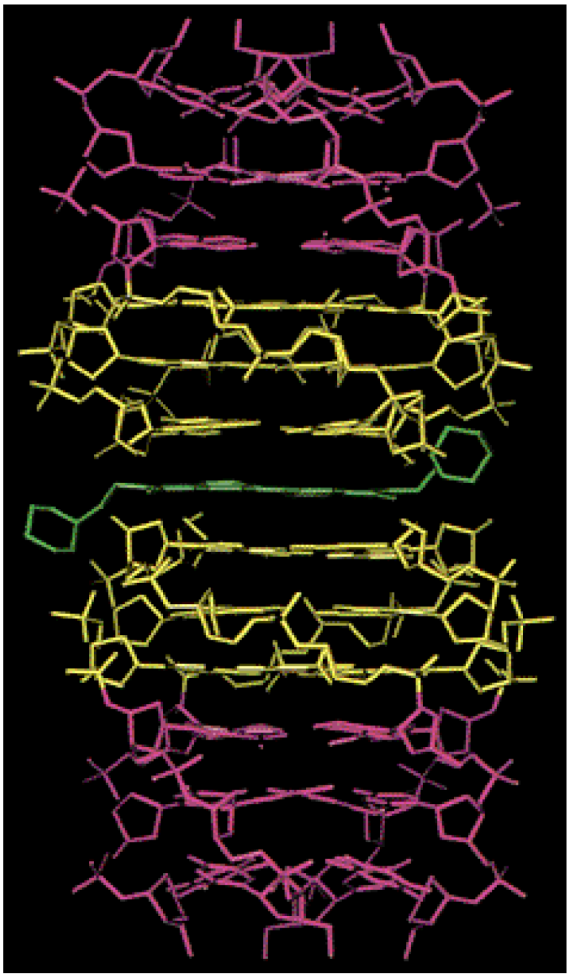


Figure 1.14 NMR based model of the 2:1 d[TTAGGG]$_4$ – PIPER complex

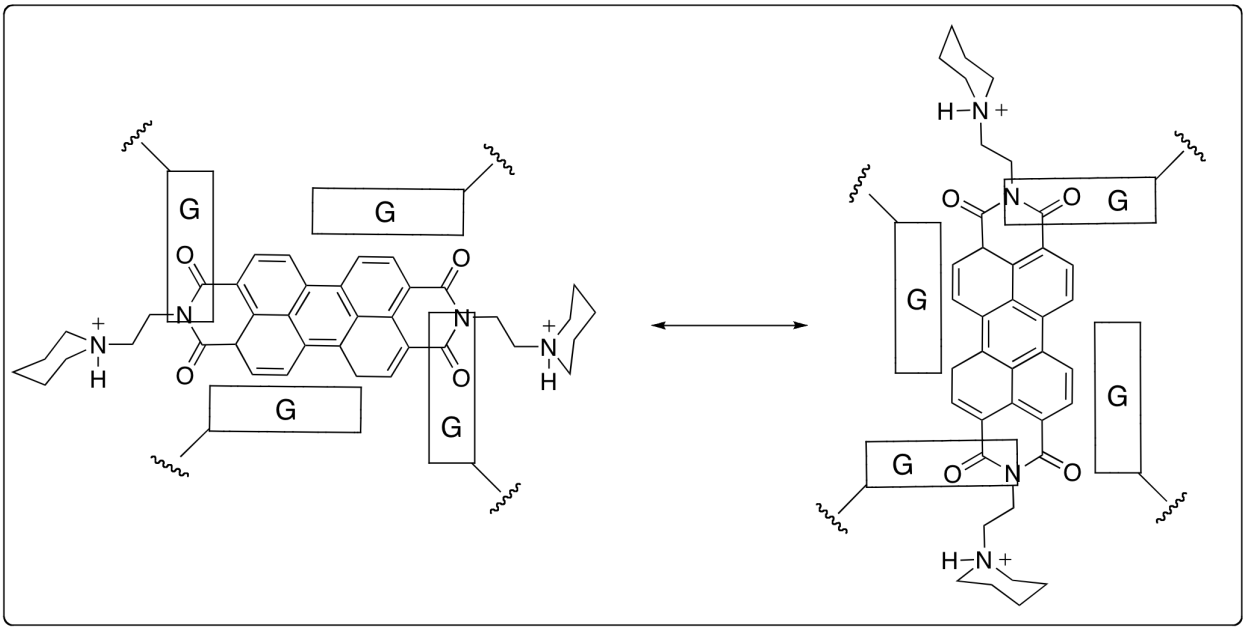


Figure 1.15 Structural transition between two orthogonal drug orientations of PIPER

Because of its two-molecule aggregation PIPER have the better selective binding to G-quadruplexes over double helical DNA structure. PIPER can also facilitate the rate of formation of hairpin bimolecular G-quadruplex by about 100 times. However the effectiveness of PIPER binding varies with pH, at lower pH conditions PIPER exists as a single molecule and has similar binding with double stranded DNA and G-quadruplexes, at higher pH conditions PIPER can aggregate and has a better selectivity with G-quadruplex structures [49].

1.5.1(b) TMPyP: Porphyrin compounds have been known since long time as ligands to bind the double stranded DNA, however the aromatic ring of porphyrins can stack on top of G-quartets. The represented TMPyP4 [50] ((*meso*-5,10,15,20-tetrakis-(*N*-methyl-4-pyridyl) porphine tetratosylate) (2) (Figure 1.16) is the first type of porphyrin molecule that have been reported to bind G-tetrad with high affinity. However its cyclic shape and pi stacking ability make it an excellent DNA binder although this ligand suffers from very poor quadruplex specificity. The

TMPyP4 ‘s isomer TMPyP2 (1) (Figure 1.16) is also binds to the G-quadruplex but it has a weaker activity of the mutual recognition of a G-quadruplex due to the steric hindrance resulting from the location of methyl groups. From the data analysis of the UV melting temperature studies shows that both TMPyP4 and TMPyP2 stabilizes the antiparallel quadruplex DNA structure to about the same extent. TMPyP4 forms the complex with d[TAGGGTTAGGG], and from its crystal structure it was evidenced that TMPyP4 only stacked on the 5’ end region of a TTA loop, and had no direct contact with G-quartets, this observation was also supported by the photocleavage experiment studies. From the NMR studies it was clear that TMPyP4 had an external π - π stacking interaction with the c-myc G-quadruplex.

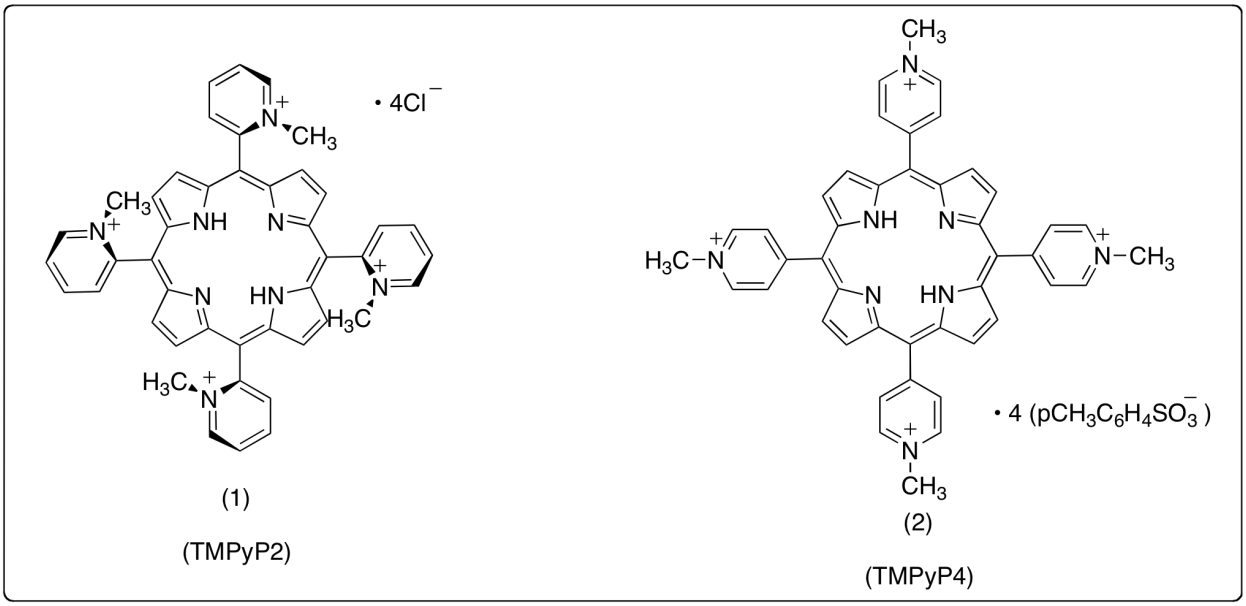


Figure 1.16 Structures of TMPyP2 and TMPyP4

Several structurally related ligands have been described over the past years: the porphyrin TQMP (4) (Figure 1.17) [51] and the porphyrazine 3,4-TMPyPz (3) (Figure 1.17) [52] are two examples of tetracationic macrocylces, which have been shown to bind efficiently to quadruplex DNA. In

particular in the case of the porphyrazine derivative, a 100-fold increase in affinity as compared to TMPyP4 has been measured by SPR, but also a significant improvement of the specific recognition of quadruplex over duplex DNA was observed.

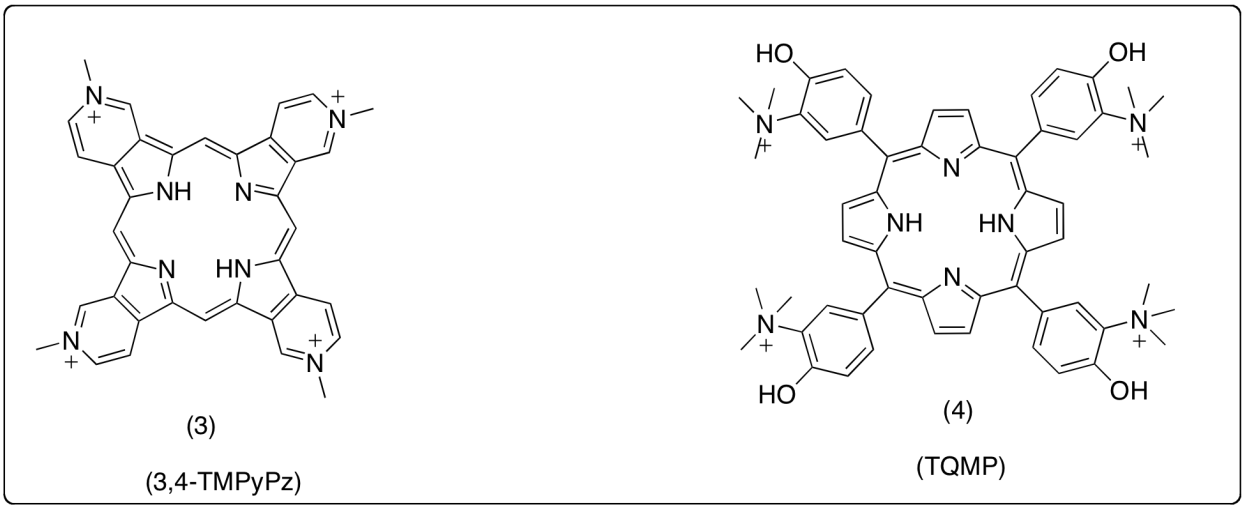
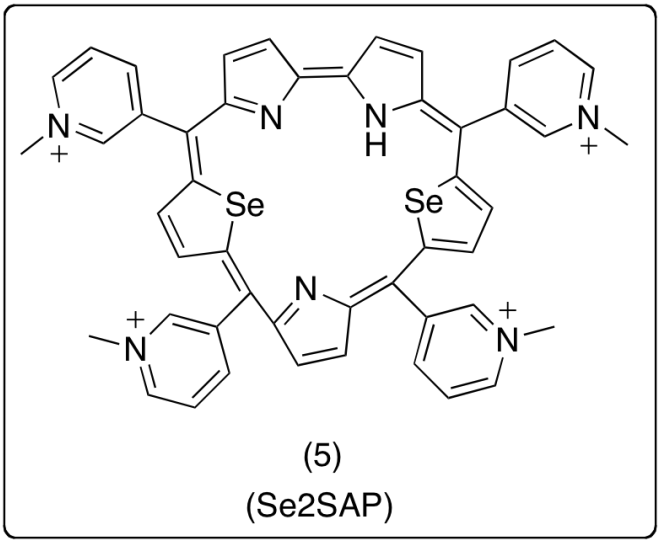


Figure 1.17 Structures of 3,4-TMPyPz and TQMP

Recently an important breakthrough in the porphyrin series came with the design of a diselenosapphyrin Se2SAP (5) (figure 1.18) with an expanded porphyrin core [53]. This ligand was shown to bind strongly and selectively to quadruplex DNA and to convert parallel (*c-myc* sequence) or anti-parallel (human telomeric sequence) topologies to a mixed anti-parallel/parallel hybrid structure.



(5)
(Se2SAP)

Figure 1.18 Structure of Se2SAP

1.5.2 Flat aromatic ring systems:

1.5.2 (a) Berberines: Berberine (6) (Figure 1.19) is an antibiotic alkaloid originating from chinese herbal medicine [54]; its antibacterial activity has been demonstrated against many species [55]. But later on it was screened for anti cancer activity following evidence of anti- neoplastic properties, these properties were arising from the inhibition of telomerase elongation [56]. Coralyne (7) (Figure 1.19) a synthetic analogue of berberine also binds to triplex DNA [57]. However from the competition dialysis experiments [58] it was found that both compounds have selectivity for triplex DNA and to a minor extent for quadruplex DNA compared to duplex DNA. Molecular modeling studies of interactions between berberine derivatives and human parallel G-quadruplex structure indicate that berberine is stacked on the terminal G-tetrad of the quadruplex (Figure 1.20). The ability of berberine and coralyne to form inter and intra molecular G-quadruplex structures was investigated by polyacrylamide gel electrophoresis (PAGE) and it showed that both analogues were able to induce G-quadruplex dimeric structures, but each to a different extent. The telomeric repeat amplification protocol (TRAP) assay was used to measure the

telomerase inhibition and from these essays coralyne shows higher telomerase activity with an IC50 value of 70 micro M and berberine shows an activity with an IC 50 value of >130 micro M.

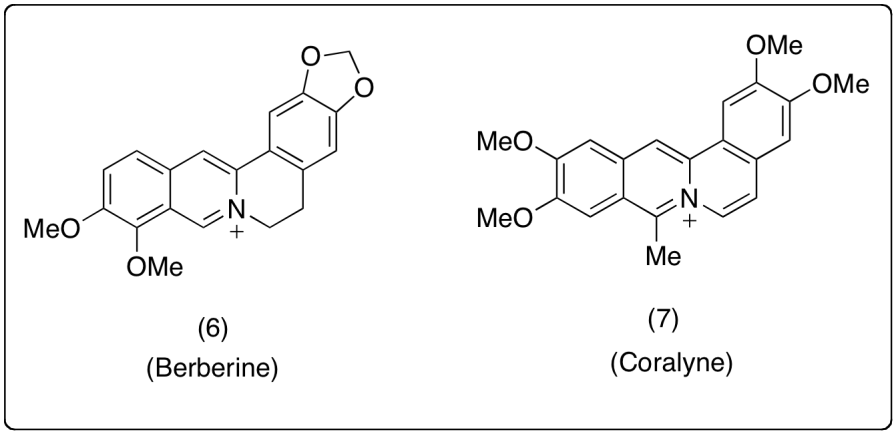


Figure 1.19 Structure of berberine and coralyne

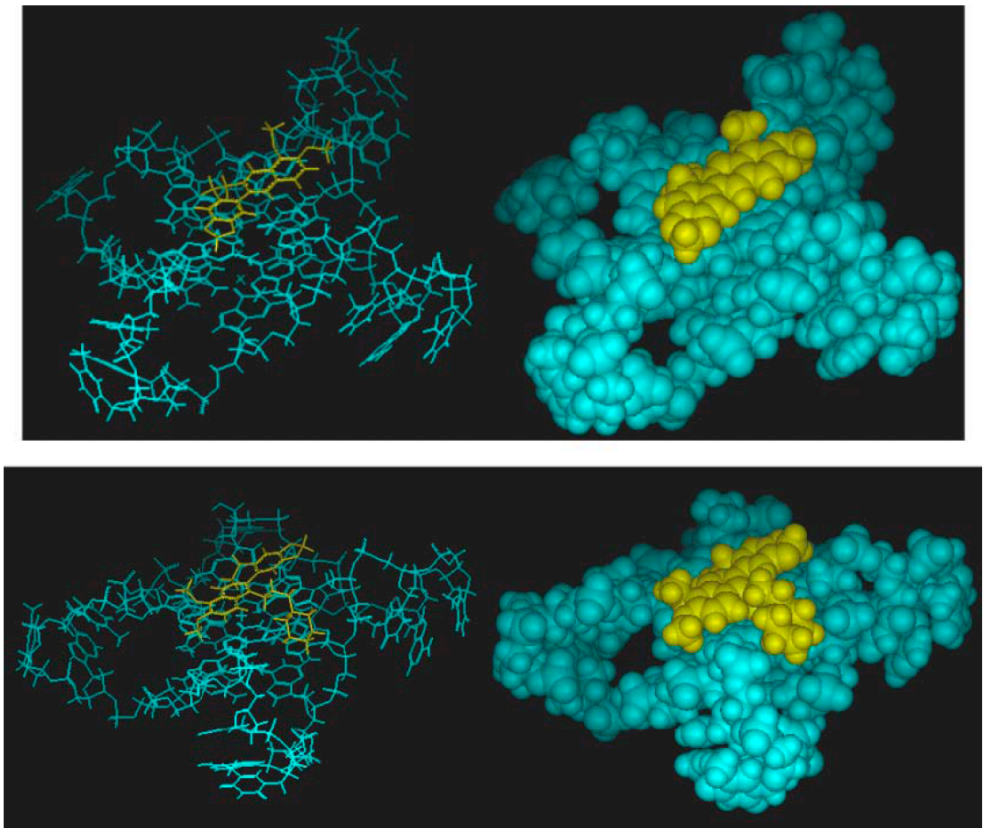


Figure 1.20 Models for the complexes of berberine (top) and piperidino-berberine (bottom) with a monomeric G-quadruplex (blue) and ligand molecules**.**

1.5.2(b) Daunomycine: It is evidenced that the cellular activity of established duplex DNA-interacting anti cancer drugs such as the anthracyclines doxorubicin and daunomycine (Figure 1.21) also involves interaction with telomeric DNA [59], possibly via quadruplex stabilization of the 31nthraquinones chromophore. The well-ordered crystal structure of a complex formed by parallel G4 quadruplex drug complex employing the anti cancer drug daunomycin was first reported by Neidle et all [60]. The complex was crystallized by the hanging –drop method in the monoclinic space group C2, with cell dimensions a = 53.078 A°, b = 47.329 A°, c = 31.914 A°, β = 119.80°.

The asymmetric unit contains four parallel d(TGGGGT) strands that form a discrete intermolecular quadruplex, together with three daunomycin molecules, 3 Na + ions and 129 water molecules. Two layers of daunomycin moleules fill the interface between two quadruplexes. The six-daunomycin molecules at the interface are arranged into two dyadrelated sets of three coplanar molecules (Figure 1.22). Each set of three daunomycins is stacked onto the 5' end of the quadruplex where they make weak π - π interactions with the guanines in the terminal tetrad. The trio of daunomycin molecules is held together in one layer by a cluster of van der walls contacts. The daunomycin layer packs tightly onto the end of the quadruplex stack, with the daunosamin sugar moieties forming H- bonding interactions and/ or van der walls contacts with three of the four quadruplex grooves.

The crystal structure also indicates that daunomycin prefers to stack onto a terminal G-quartet rather than intercalate between the layers of the quadruplex.

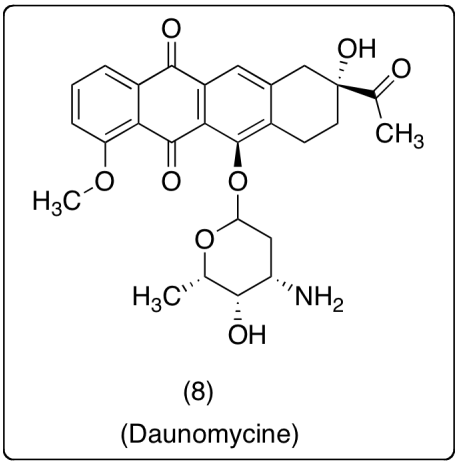


Figure 1.21 Structure of daunomycin

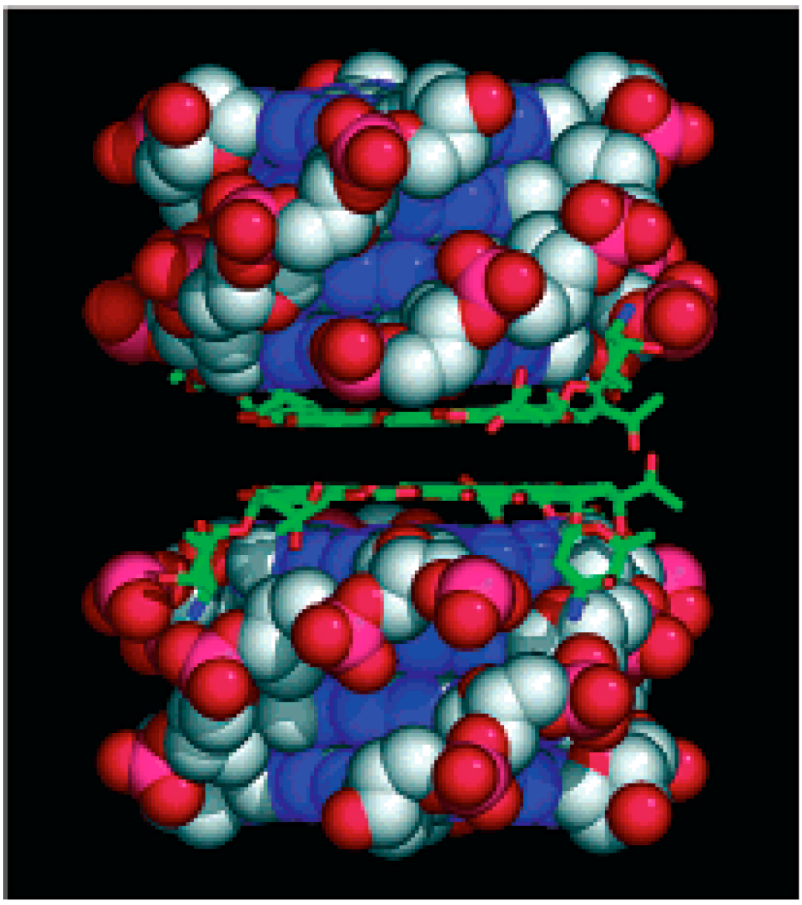


Figure 1.22 Structure of the daunomycin-d(TGGGGT) complex showing the arrangement in the crystal lattice of two quadruplexes, in vander Walls space-filling mode, and stacked end-t-end.

1.5.2(c) Fluoroquinoanthroxazines:

The fluoroquinolines are well known for their antimicrobial activity that arises by the inhibiting bacterial DNA gyrase [61]. However some tetracyclic quinoline (Figure 1.23) analogues have shown good anti neoplastic activity by topoisomerase II poisoning and by telomeres G-quadruplex stabilization.

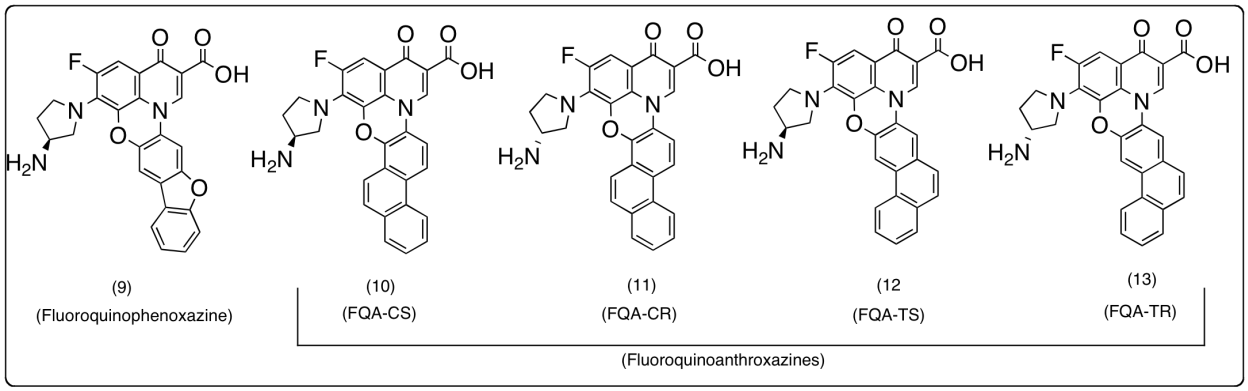


Figure 1.23: Structures of fluoroquinoanthroxazines

The binding studies of FQAs with telomeres quadruplex was reported by Hurley et all [62]. The binding affinity of the FQAs with the intramolecular G-quadruplex structures, were determined by incubating the DNA template containing four repeats of the human telomeric sequence TTAGGG with increasing concentrations of FQAs in the presence of Taq DNA polymerase. From these studies it was cleared that FQA-CR showed a modest but selective stabilization of the G-quadruplex structure. To understand even more, they also did the binding studies with *Tetrahymena* telomeric sequence, which has four consecutive guanines in each telomeric sequence (TTGGGG). From these binding studies they got the IC_{50} values of FQA-CS, FQA-CR, FQA-TS, and FQA-TR as 0.67, 0.06, 5.7, and 2.4 μM respectively. From the IC_{50} graph it was clear that FQA-CR has selectivity of about 90 fold for g-quadruplex structures over single and /or double stranded DNA (Figure 1.24).

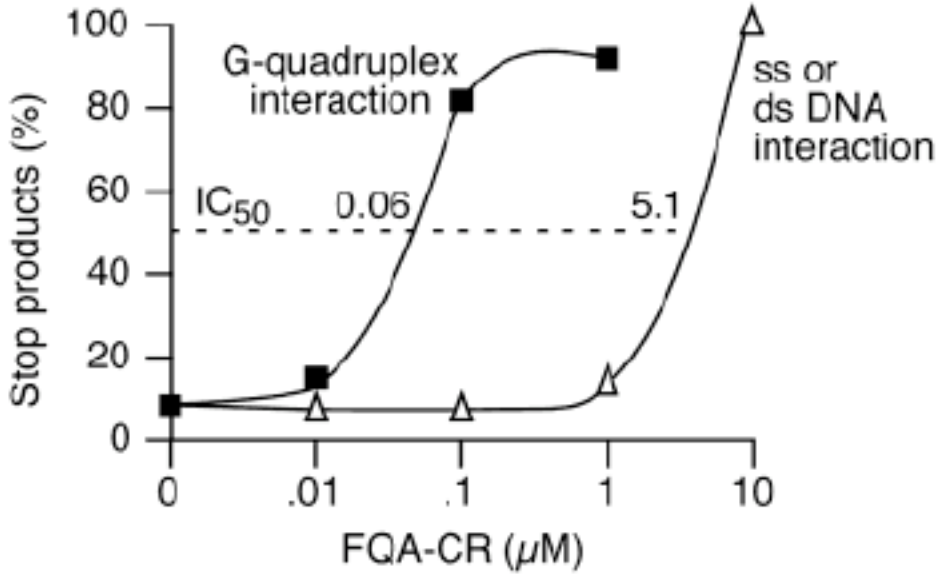


Figure 1.24 Graphical representation of the quantification of the stop products caused by the interaction of G-quadruplex or double stranded DNA with FQA-CR.

To identify the binding position of FQAs with Quadruplex, they did the photomediated cleavage reaction studies and on the basis of the results they proposed a model in which two FQA molecules selectively bid to the intramolecular chair type G-quadruplex structure through an end stacking binding mode (Figure 1.25).

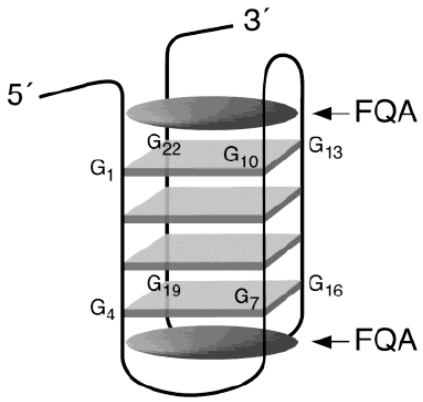


Figure 1.25 Proposed model of FQAs binding to the intramolecular chair-type G-quadruplex structures.

1.5.3 Macro cyclic Ligands:

1.5.3(a) Telomestatin and its derivatives:

Telomestatin (Figure 1.26) is a natural product isolated from streptomyces 3533-SV4 in 2001 by Shinya's group, and has been shown to be a potent telomerase inhibitor [63]. The structural similarity between telomestatin and G-tetrad suggested that the telomerase inhibition might be attributed to the ability of telomestatin to interact directly with G-quadruplex structures. The formation of a G-quadruplex structure is a slow process and takes several hours in the presence of high concentrations of monovalent cations (Na, K) [64]. However, telomestatin is able to facilitate the formation of and /or stabilizes the preformed G-quadruplex structure within one minute.

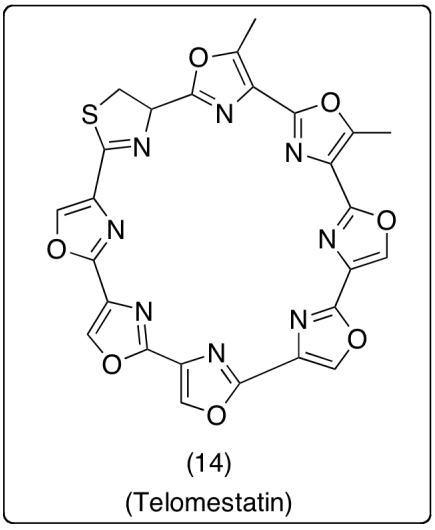


Figure 1.26 Structure of Telomestatin

Telomestatin prefers the intramolecular, rather than the intermolecular G-quadruplex structure and also it is quite selective for the G-quadruplex structure over a single stranded or duplex DNA structure. Polymerase stop assays demonstrate that telomestatin has a 70 fold high selectivity for G-quadruplex structures over single and/or double stranded DNA (Figure 1.27). Moreover, once

telomestatin binds to intramolecular G-quadruplex structures, it is not easily displaced. It has been demonstrated that telomeric function is more likely depend on structure, rather than on length alone. The maintenance of the normal telomere structure is important for cell survival. The selective interaction of telomestatin with intramolecular G-quadruplex structures would also be anticipated to have an influence on telomeric structure. This can be attributed from the sequestration of the single stranded 3'- overhangs of telomeres as an intramolecular G-quadruplex structure would prevent the formation of appropriate telomeric structures, such as T-loops.

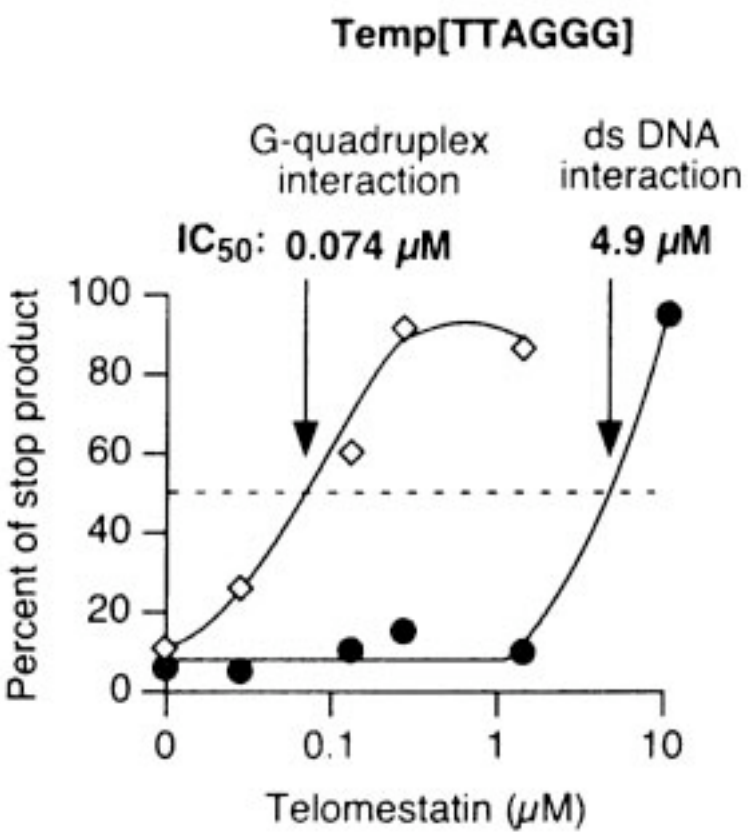


Figure 1.27 Polymerase stop essays of telomestatin

According to literature reports telomestatin accelerates the rate of telomere shortening to a greater extent than was expected from the number of population doublings, and this is

accompanied by cell growth arrest and senescence- associated morphological changes [65]. The effect of telomestatin on the activities of both telomerase and SI nuclease and similar DNA nucleases may play a key role in accelerated telomere shortening in cancer cells. Based on the results from polymerase stop assay, it was clear that the specific binding of telomestatin with intramolecular G-quadruplex structures causes the inhibition of DNA polymerase processivity at the human telomeric sequence, which might be an additional mechanism for accelerated telomere shortening.

Telomestatin interacts with the intramolecular G-quadruplex; contradictory to this TMPyP4 interacts with the intermolecular G-quadruplex. To investigate the relative importance of these two different types of G-quadruplex interactions in producing the overall biological activity, the cytotoxicities of telomestatin and TMPyP4 were determined against telomerase transformed (SW39) and ALT- transformed (SW26) cell lines respectively. These cells maintain their telomeres either through the telomerase (telomerase- positive) and alternative lengthening of telomeres (ALT-positive) mechanisms. From the fig the IC_{50} values are found to be 4.1 μM (telomestatin against SW39), 1.8 μM (telomestatin against SW26), 56.3 μM (TMPyP4 against (SW39) and 62.9 μM (TMPyP4 against SW26) (Figure 1.28) [66].

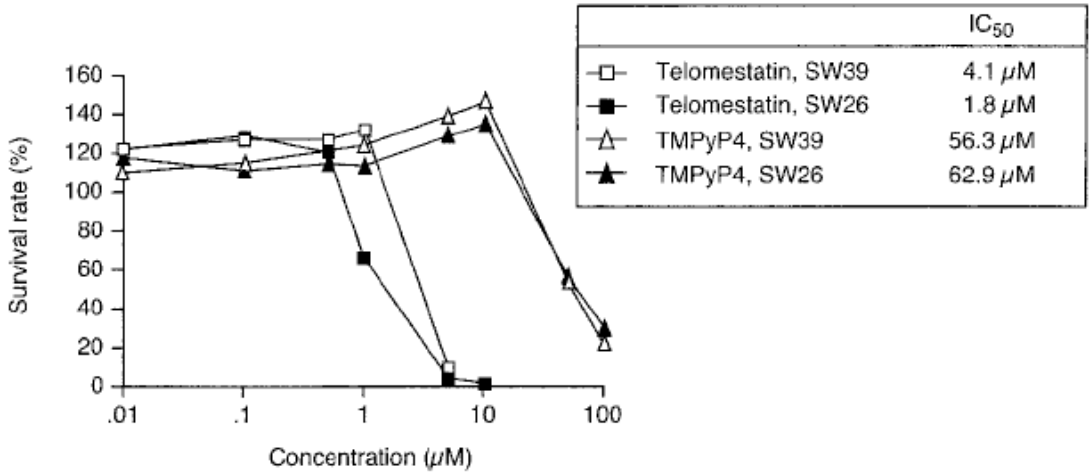


Figure 1.28 Cytotoxic assays of telomestatin and TMPyP4

Getting the inspiration from the binding modes of telomestatin, Nagasawa et all synthesized some telomestatin derivatives such as 6OTD which has C_2- symmetrical macrocyclic hexaoxazole structure, as a new G-quadruplex binder (Figure 1.29) [67].

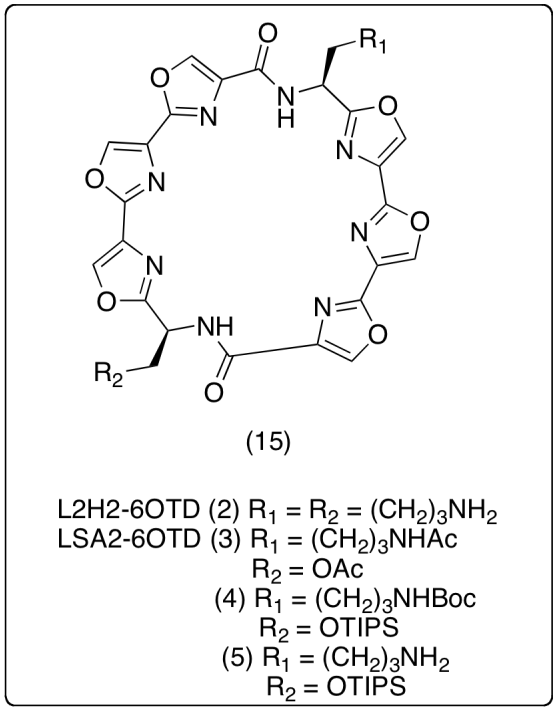


Figure 1.29 Chemical structure of 6-OTDs

By considering the proposed stacking model of telomestatin with telomeric G-quadruplex, they hypothesized that a 6OTD dimer connected through an appropriate linker would show cooperative interaction of the two monomer moieties with telomeric DNA, and would therefore bind more selectively than the monomer (Figure 1.30) [68].

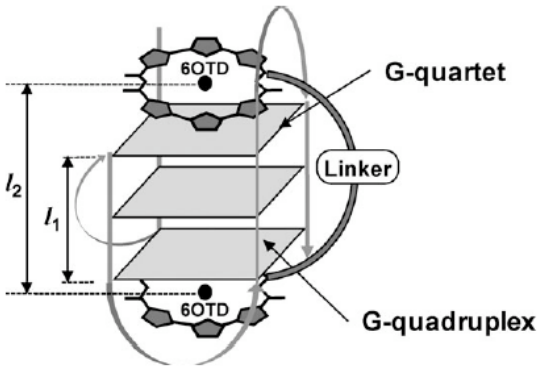


Fig. 2 Design concept of 6OTD dimers.

Figure 1.30 Design concept of 6OTD dimers

1.6 Metallo-Organic G-quadruplex ligands

The use of metallo-organic complexes became a very good alternative to the use of classical organic molecules. This class of ligands is highly interesting, because of their easy synthetic access and their very promising G-quadruplex binding properties.

This approach is based on the assumption that the central metal centre could be positioned over the cation channel of the quadruplex, there by optimizing the stacking interactions of the surrounding chelating agent with the accessible G-quartet [69]. The cationic or highly polarized

nature of these complexes also promotes the association with the negatively charged G-quadruplex-DNA.

The first reported examples of this kind were started with the insertion of metal atoms such as Cu(II), Ni(II) or Mn(II) in the cavity of TMPyP4 (Figure 1.31) [70]. Among all these Mn-TMPyP4 showed a 10 –fold preference for quadruplex over duplex DNA [71]. The other transition metal complexes like Ru(II) [72], Fe(III) [73], Zn(II) [74], Pt(II) [75], Ni(II) –Salphen [76] and Mn (III)-Porphyrin [77] were appeared to stand amongst the most potent reported G-quadruplex ligands. Their performances are indeed impressive both in terms of quadruplex stabilization and quadruplex selectivity that were evaluated by FRET –melting assay and SPR. These compounds also display good level of telomerase inhibition (IC_{50}-TRAP= 120 and 580 nM for Ni(II) and Mn(III)-complexes respectively. The Mn(III) –porphyrin complex also showed a 10000-fold quadruplex *vs.* duplex selectivity measured by SPR [78].

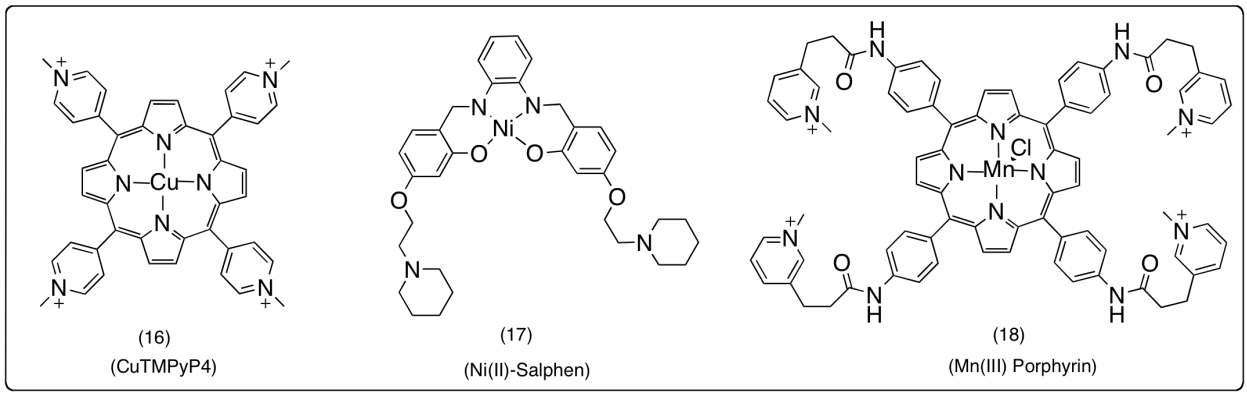
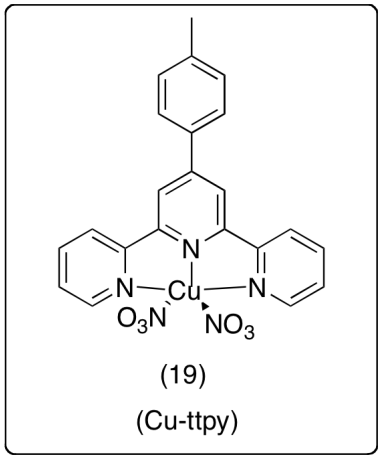


Figure 1.31 Structures of Metallo-Organic G-quadruplex ligands

The very simple structures such as Cu(II) (Figure 1.32) and Pt(II)-terpyridine complexes that can be synthesized in one-step or two-step processes have proved to be high-affinity and highly

selective G-quadruplex ligands [79]. All these studies highlighted that the geometry of the metal centre is a key parameter governing the selectivity.



(19)

(Cu-ttpy)

Figure 1.32 Structure of Cu-ttpy

1.7 Aim of the Dissertation

From the literature survey it was clear that, the small ligands that stabilize the G-quadruplex formed by the guanine rich telomeres can inhibit the interaction between telomeres and telomerase. This inhibition ultimately leads to the cell death. The selective activity of telomerase in cancer cells makes this approach as versatile to develop a new class of anti cancer drugs.

The dissertation will focus on the following goals like design and synthesis of small ligands that stabilize the G-quadruplex structure. The synthesis of the ligand molecules must start from the easily available starting materials and the over all synthesis should be easily accessible and applicable to large-scale manufacturing. The main focus should be synthesizing a diverse library of compounds and evaluate them as anti cancer drugs on various types of cancer cell lines.

1.8 References and notes:

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Chapter-2: Synthesis and Anti-Cancer Evaluation of Acridine Derivatives

2.1 Introduction:

2.1.1 Acridines: Acridine is a naturally occurring anthracene like heterocyclic containing a nitrogen atom on its central ring. It was isolated in 1871 by Graebe and Caro from coal tar.

Acridone (acridin-9(10H)-one) is the ketone derivative from acridine. The first identified synthesis of acridone was the oxidation of acridine and was reported by Graebe and Caro in 1880.

Acridine and acridone analogues have been known since long time for their various biological activities such as anti bacterial drugs (1-6) [1], anti protozoal drugs (7-12) [2], anti malarial agents (13) [3], and anti HIV drugs (14) [4].

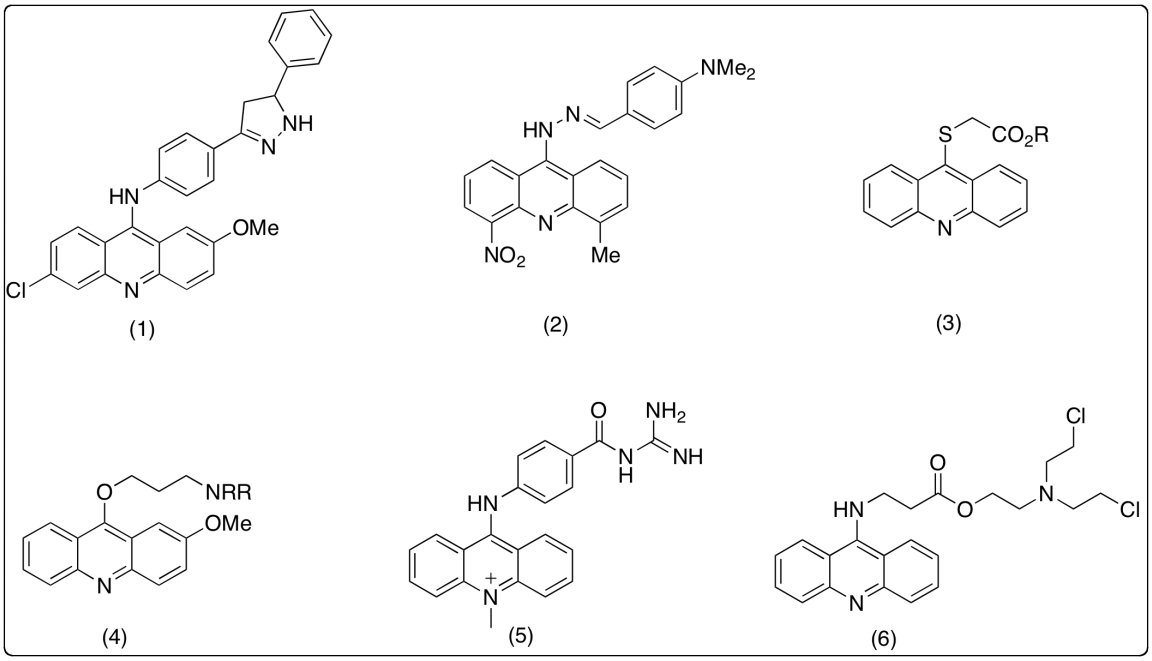


Figure 2.1 Acridine based anti bacterial drugs

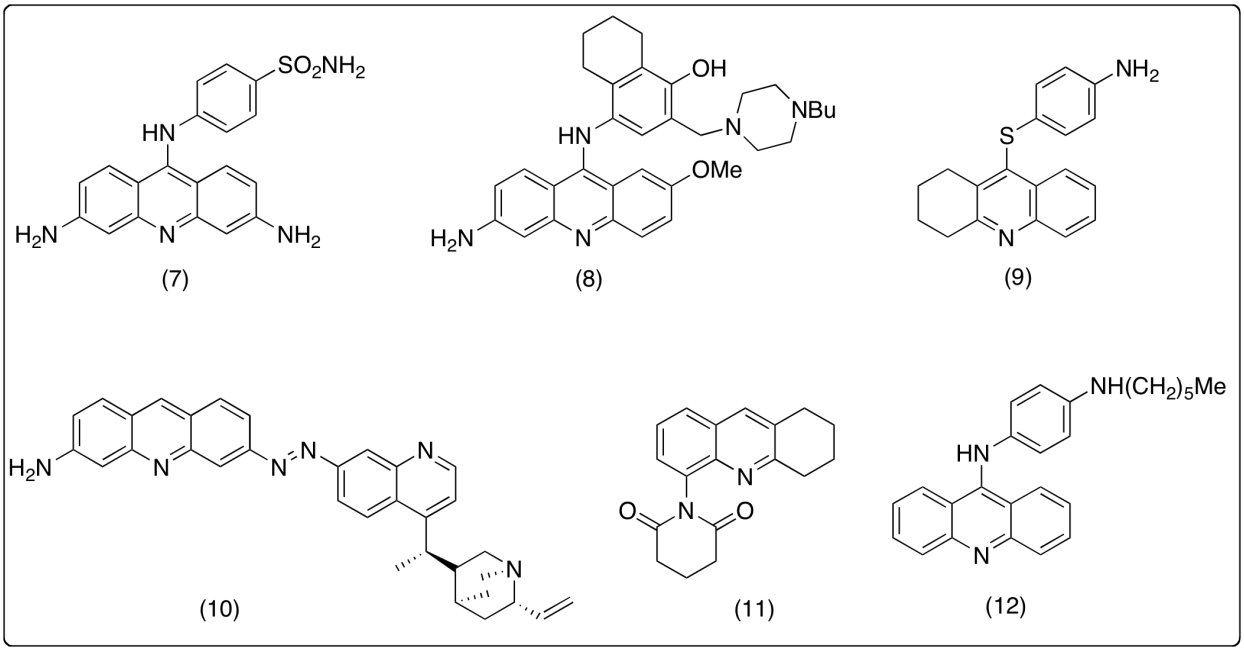


Figure 2.2 Acridine based anti protozoal drugs

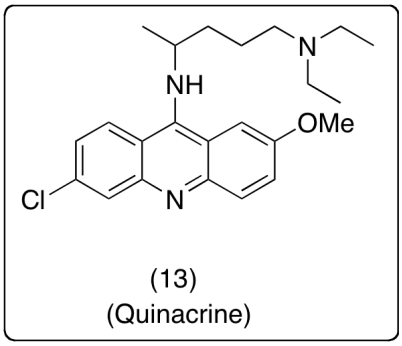


Figure 2.3 Acridine based anti malarial agents

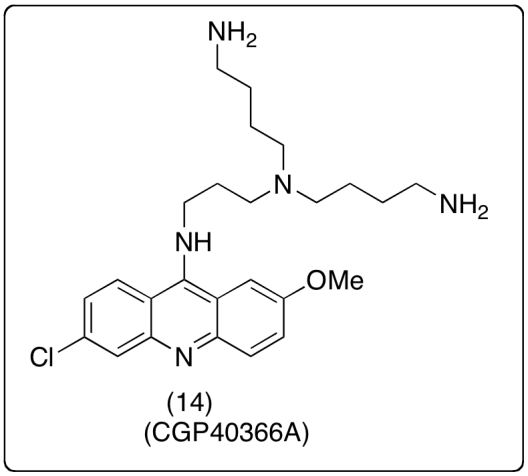


Figure 2.4 Acridine based anti HIV drugs

However many acridine and acridone derivatives such as asulacrine analogues (15,16), acridine carboxamides, e.g., N-(2-(dimethylamino)ethylacridine-4-carboxamide (DACA) (17); nitro

acridines (18), nitropyrazolo-acridine (19), bis –acridines (20) and amsacrine (21) also shown the anti cancer activity [5]. We also have several natural acridine/acridone analogs of alkaloids that are isolated from plants and marine organisms can exhibit the anti cancer activity [6].

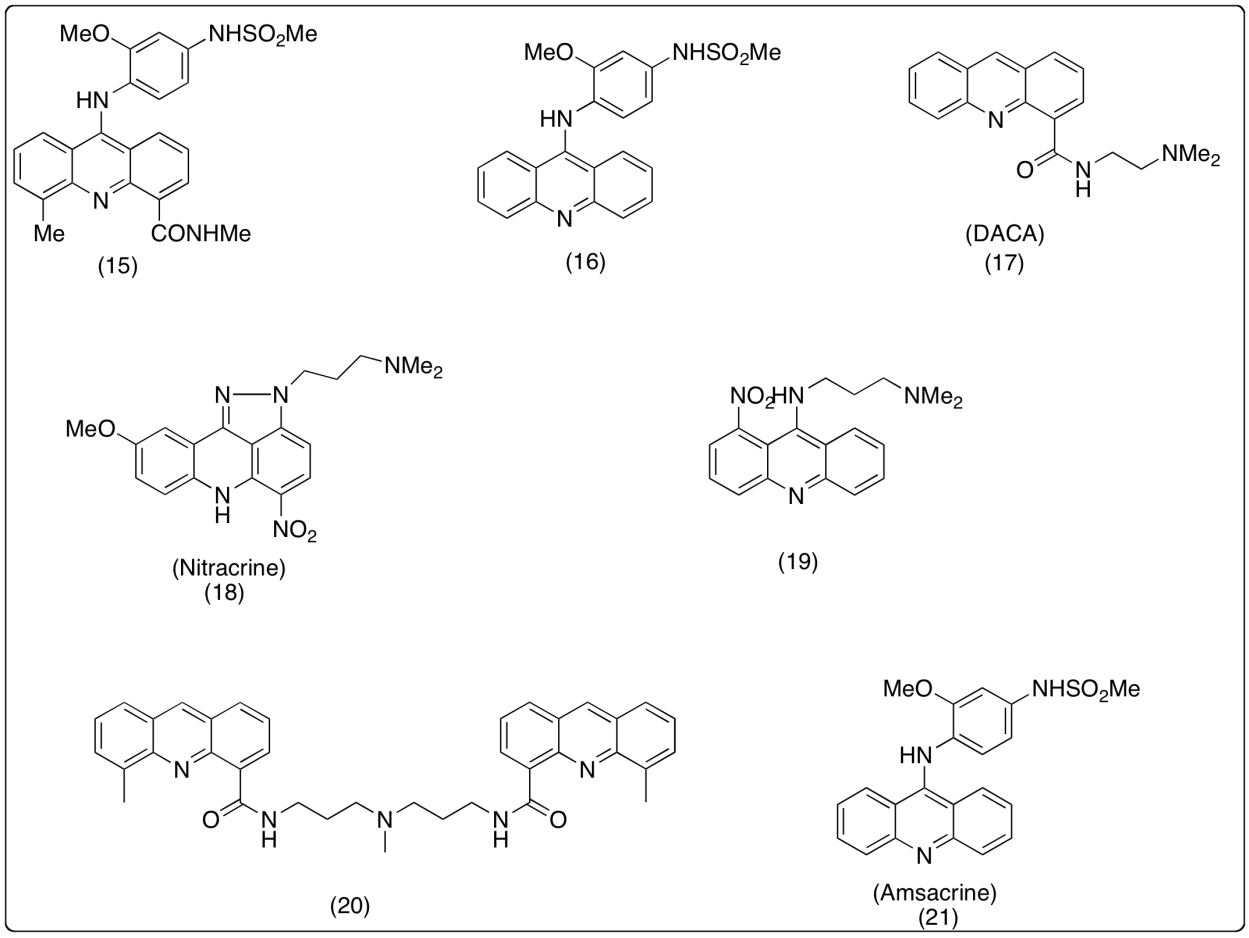


Figure 2.5 Acridine based anti cancer analogues

The anti cancer activity of acridine/acridone derivatives arises from their capability to intercalate with DNA and inhibit topoisomerase or telomerase enzymes [7].

2.1.2 Acridine/Acridone as DNA targeting Agents:

The utility of acridines as chemotherapeutics is due to their chemical and biological stability and their capability of effective binding to DNA or RNA [8], resulting in the disorder of the biological functions in living cells. The mechanism of their intercalation into DNA is based on π-stacking interaction with base pairs of double-stranded nucleic acids. The heterocyclic, polyaromatic flat structure of acridine fits effectively into the gap between two chains of polynucleotides and the intercalation of the acridine moiety disturbs their crucial role in cell division [9].

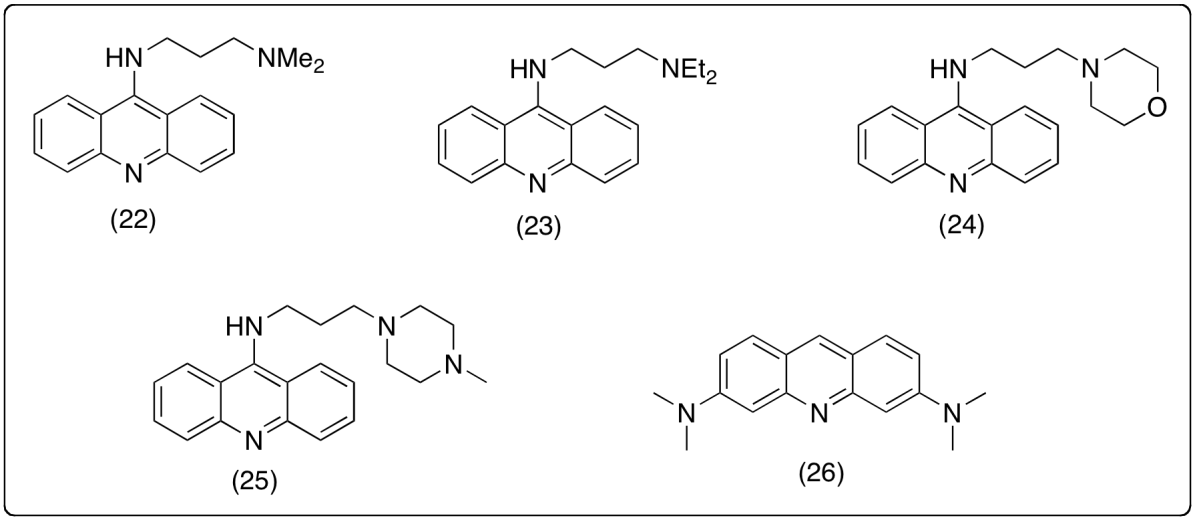


Figure 2.6 Acridine based DNA targeting agents

2.1.3 Acridines as topoisomerase inhibitors:

DNA topoisomerases are a class of enzymes involved in the regulation of DNA supercoiling [10]. There were 2 types of topoisomerases, type I topoisomerases change the degree of supercoiling of DNA by causing single-strand breaks and religation, where as type II topoisomerases cause double strand breaks. Even though topo I and II may indicate opposing roles in the regulation of

DNA supercoiling, both activities are necessary during DNA transcription, replication and chromatin condensation.

Amsacrine (*m*-AMSA), obtained by Denny's group was the first synthetic drug that was shown to act as a topoisomerase inhibitor and that was approved for clinical usage [11]. It has been used in leukemia treatment since 1976. The other series of acridine derivatives, like anilinoacridines, and acridin-4-carboxamides were also interfere to some extent with topoisomerases. Among these DACA, which was prepared in 1987 is one of the exceptional compounds that inhibit both topo I and II isomerases [12].

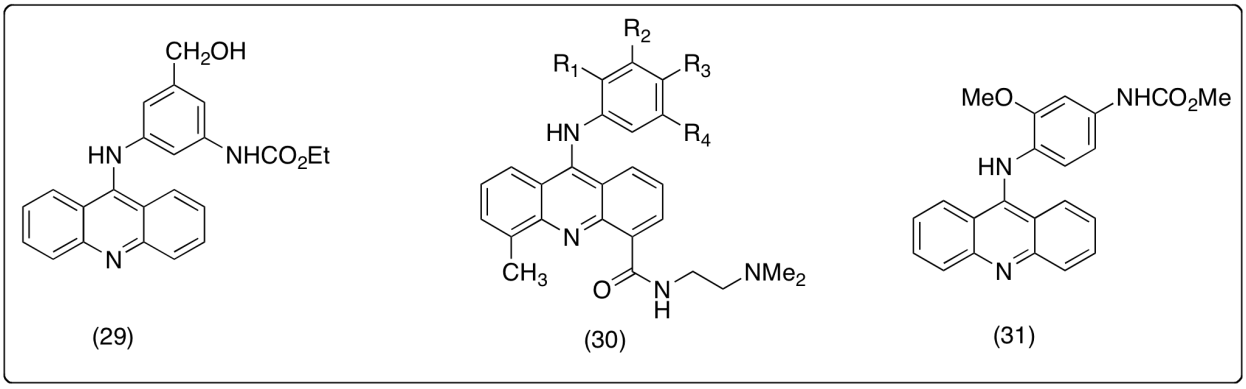


Figure 2.7 Acridine based topoisomerase inhibitors

2.1.4 Acridines as telomerase inhibitors:

Several acridine based small molecules have been described to inhibit telomere maintenance via the stabilization of the quadruplex G4 structure, thus inhibiting the telomerase action [13]. A number of studies have demonstrated that the inhibition of telomerase in cancer cells leads to senescence and apoptosis. Among these studies, there are some acridine-based structures, which can be divided into three sub-families;

1) Dibenzophenanthrolines
2) Pyridoacridines
3) Trisubstituted acridines.

2.1.4(a) Dibenzophenanthrolines:

The quinacridine class of acridine based quadruplex-stabilizing agents; dibenzophenanthrolines were developed in 2001 by Teulade-Fichou [13]. These pentacyclic quinacridines that display a crescent shape likely to maximize the overlap with the guanines of the accessible G-quartet.

Among all of quinacridines family, MMQ_3 was the leading compound. It shows remarkable G-quadruplex stabilization ($\Delta T_{1/2}$ = 20 °C) and high telomerase inhibitory activity (IC_{50}-TRAP = 28 nM). The NMR structure was available with MMQ_1, the dipropylamino analogue of MMQ_3, and a tetramolecular quadruplex (Figure 2.9) [14]. This study not only shows the simultaneous overlap of three guanines by the quinacridines unit, but also pinpointed the role of the protanated sidearms, which actively participate in quadruplex recognition via interaction in the grooves. A dimeric macrocyclic quinacridine was subsequently proposed, BOQ_1, that proved to be an improved quadruplex stabilizer (IC_{50}- TRAP = 130 nM) [15]. This selectivity attributed to the enhancement of the ligand aromatic surface, is also likely a consequence of the steric hindrance of the macrocyclic scaffold that imedes duplex binding.

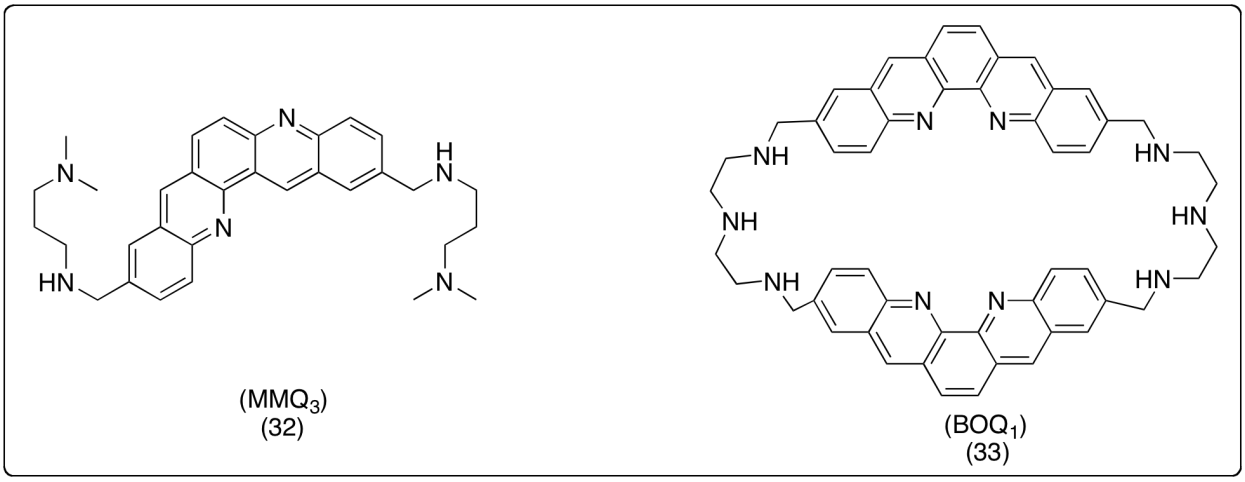


Figure 2.8 Quinacridine based telomerase inhibitors

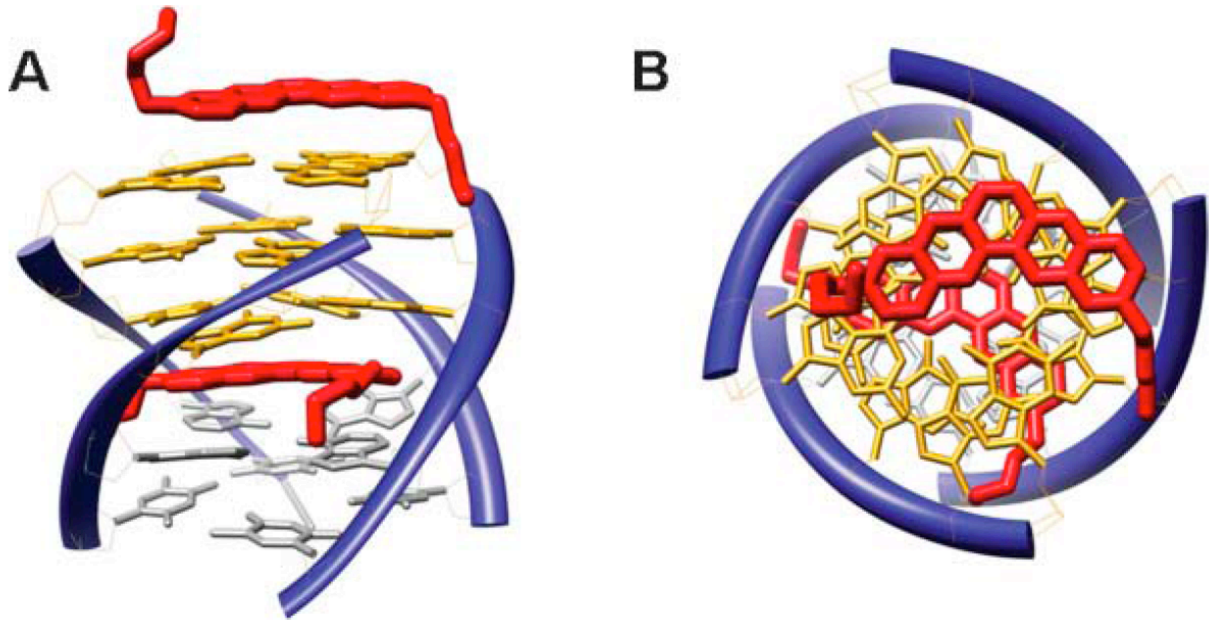
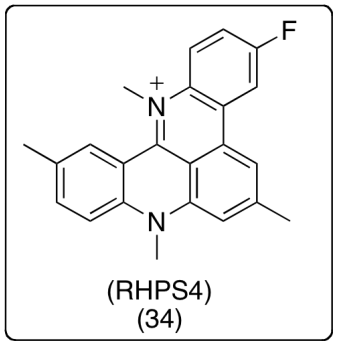


Figure 2.9 Side- (A) and top-views (B) of the NMR structure of MMQ_1 complex with tetramolecular quadruplex-DNA $(d[T_2AG_3T])_4$ (PDB entry: 2JWQ)

2.1.4(b) Pyridoacridines:

The Stevens group reported RHPS4, a *N*-methylated pentacyclic acridinium that stabilizes the G-quadruplex in 2000 [16]. *In vitro* studies (IC_{50}- TRAP = 330 nM) and *in cellulo* investigations demonstrated the ability of this highly condensed aromatic ligand to decrease telomere length and to act in synergy with the classical anti-cancer agent Taxol. Recently, RHPS4 has also been reported as an efficient telomere uncapping agent, as well as a telomere binding proteins modulator [17]. It is important to mention that RHPS4 is one of the rare ligands whose complex with G-quadruplex-DNA has been solved by NMR (Figure 2.11). The cationic molecule sandwiches the quadruplex- structure because of strong stacking interactions between the ligand and the two external G- quartets of the G-quadruplex.



(RHPS4)
(34)

Figure 2.10 Pyridoacridine based (RHPS4) telomerase inhibitor

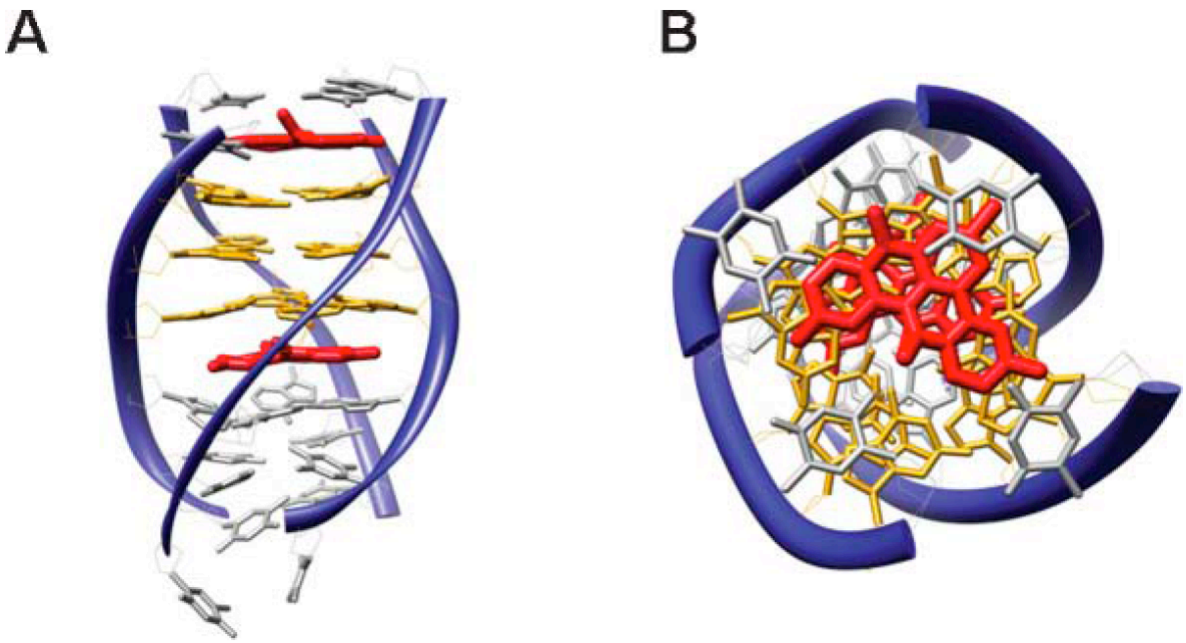


Figure 2.11 Side- (A) and top-views (B) of the NMR structure of RHPS4 complex with tetramolecular quadruplex-DNA $(d[T_2AG_3T])_4$ (PDB entry: INZM)

2.1.4(c) Trisubstituted acridines:

The key issue in the development of compounds that target G-quadruplex –DNA is to conceive a large flat aromatic system prone to π- stacking with G-tetrad platform, while retaining reasonable water solubility. In other words, the molecule has to exhibit both hydrophobic and hydrophilic characteristics. A usual way to ensure this duality is to introduce protanable sidearms like amine groups around an aromatic core; the molecule is then, water soluble, with the charges far from the hydrophobic centre.

By considering all these information into account, Prof Neidle group designed a 3,6,9-trisubstituted acridine-based molecules that selectively interact with the human DNA quadruplex by using computer modeling [18].

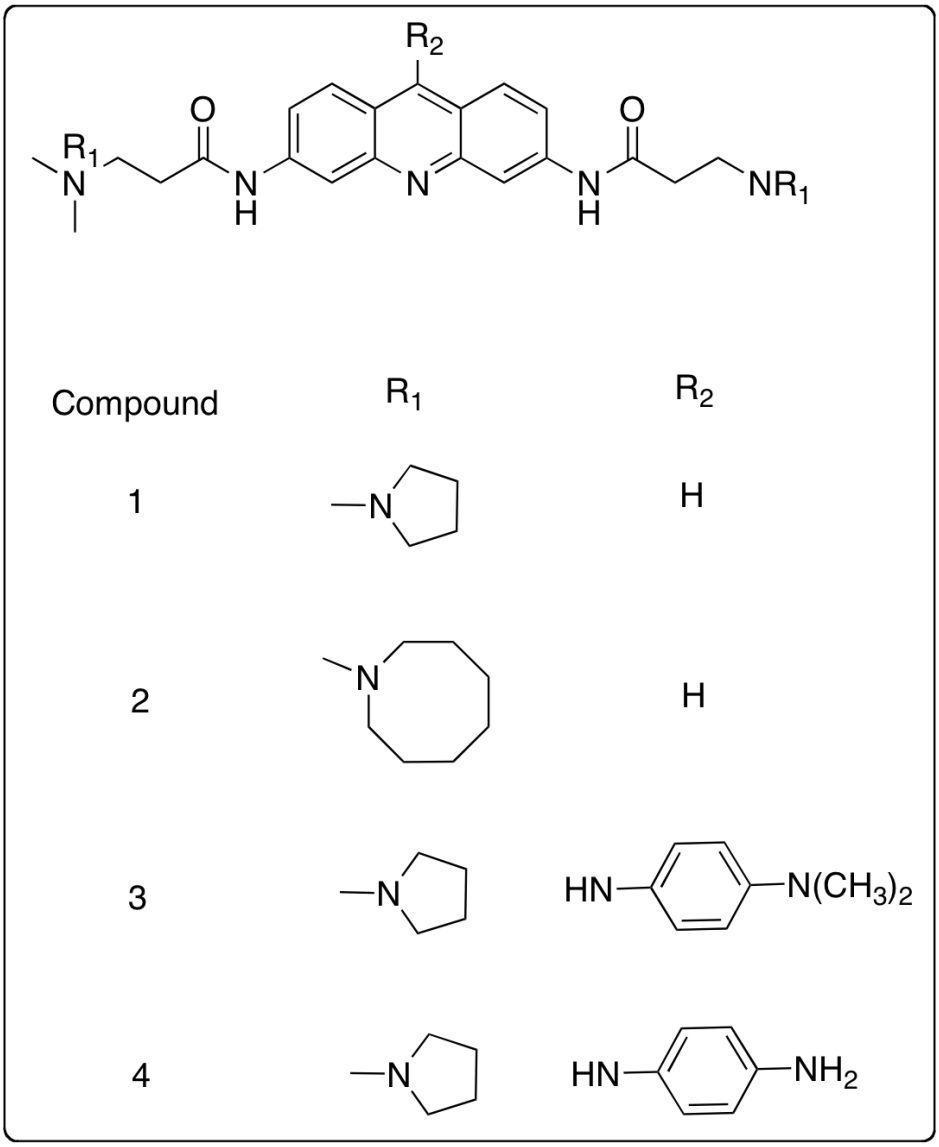
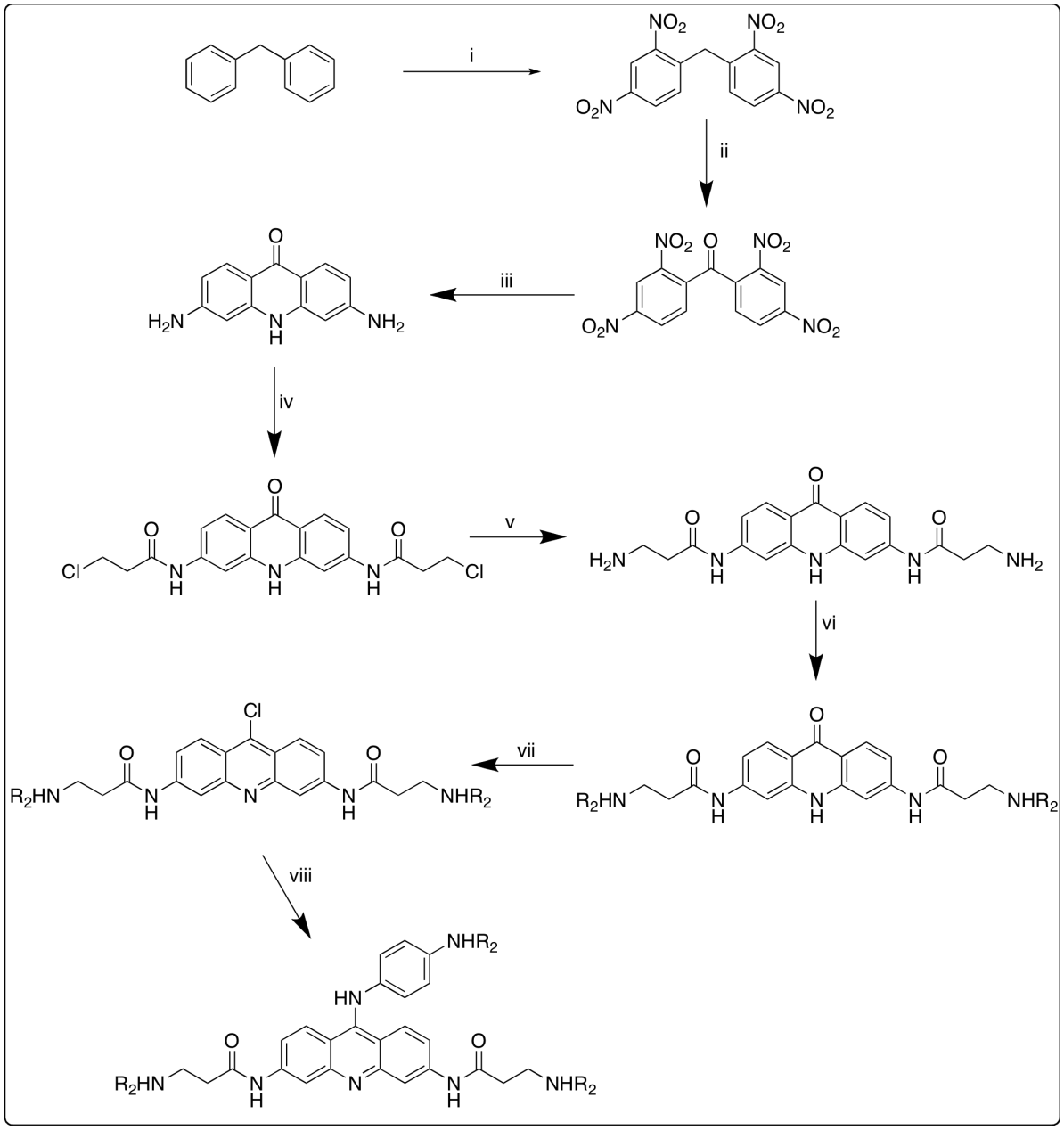


Figure 2.12 Trisubstituted acridine based telomerase inhibitors

2.1.4(d) Synthesis of the trisubstituted acridine derivatives:

Compounds were synthesized as shown in the scheme



Scheme 2.1 Synthetic scheme for the 3,6,9-trisubstituted acridine derivatives. The individual steps involved (i) KNO_3, H_2SO_4 (ii) CrO_3, AcOH, Reflux (iii) Zn/ HCl, 90-100°C (iv) 3-CPC, Reflux (v) NHR_2, EtOH, NaI Reflux (vi) HCl (vii) $POCl_3$, Reflux and (viii) NH_2PhNR_2, $CHCl_3$, RT

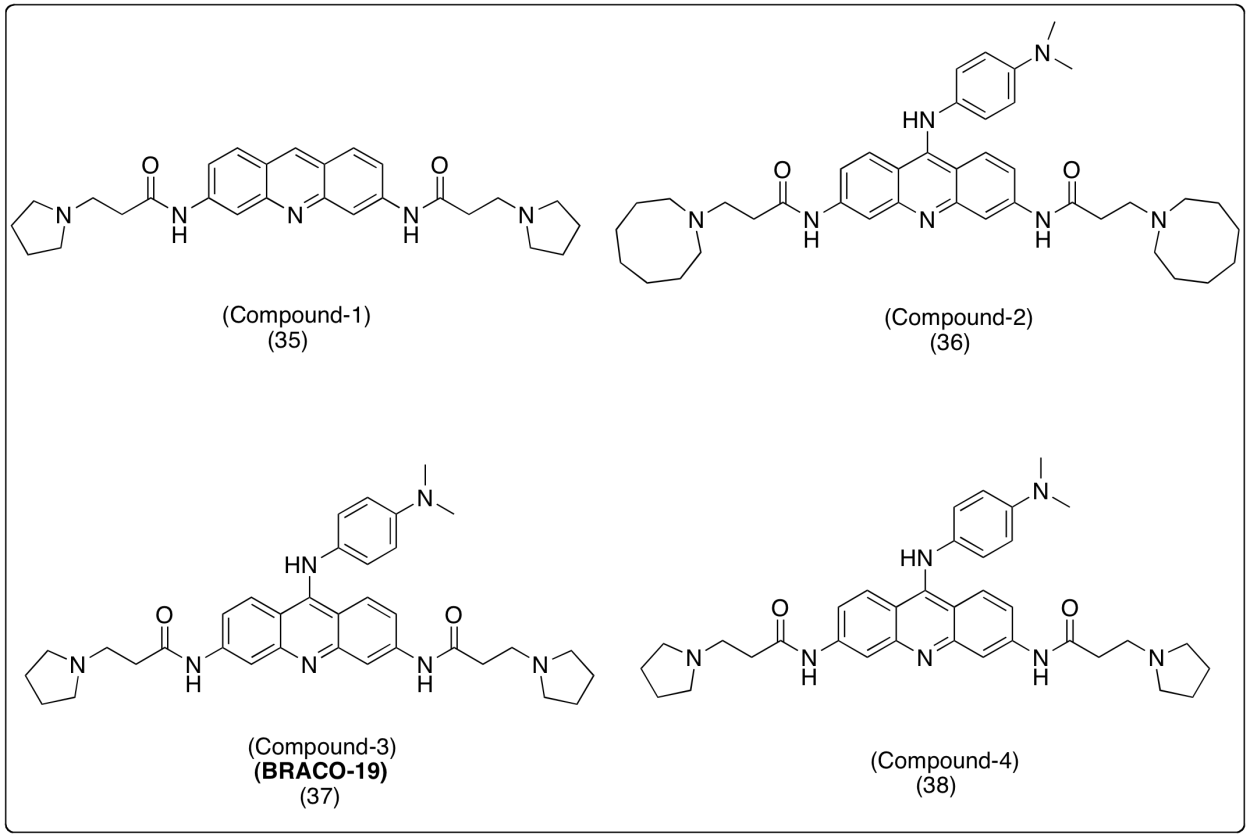


Figure 2.13 Prepared trisubstituted acridine derivatives

The binding of all the ligands to the human quadruplex and a representative duplex structure was examined by SPR techniques. The SPR data of all compounds indicates that the disubstituted compound 1 has approximately the same binding constant for duplex and quadruplex, where as the two trisubstituted compounds 3 (37) and 4 (38) bind to human quadruplex DNA 30-40 times more strongly than to duplex. Their affinity for the quadruplex is 10-fold higher then that of compound 1 (35).

However among all these the compound 3 (BRACO-19) (37) is very well studied.

Figure 2.14 The biological unit in the crystal (PDB id 3CE5). BRACO-19 molecule (mauve) is shown at the interface of the two quadruplex in the unit, stacked between a G-quartet (top) and a TATA tetrad (bottom).

The TRAP assays show us that the activity of disubstituted acridine, compound 1 (35) is typical of the more active anthraquinones. Compound 2 (36) is essentially inactive as a telomerase inhibitor. However the two-trisubstituted compounds 3 (37) and 4 (38), showed telomerase activity at levels up to 100- fold greater potency. Both are also significantly less potent in the cytotoxicity assay; with compound 4 (BRACO-19) (36) having outstandingly low activity (as well as being the most potent telomerase inhibitor (table 2.1)).

Table 2.1 Telomerase inhibition, and cytotoxicity, given as EC_{50} and IC_{50} values of trisubstituted acridine derivatives in μM

Compound	$^{tel}EC_{50}$	A2780 IC_{50}	CH1 IC_{50}	SKOV-3IC_{50}
1	5.2	2.65	8.2	2.6
2	>50	1.3	2.2	2.3
3	0.095	10	10.1	13
4	0.06	>25	>25	>25

BRACO-19 has a good solubility of at least 2mg/ml in water and in physiological pH conditions.

However, its very poor permeability is its main biopharmaceutical limitation [19].

2.2 Results and Discussion:

With consideration of BRACO-19 and tri substituted acridine derivatives along with their advantages and disadvantages, we rationally design a general structure of target molecule (Figure 2.15).

2.2.1 General Structure of the target molecule:

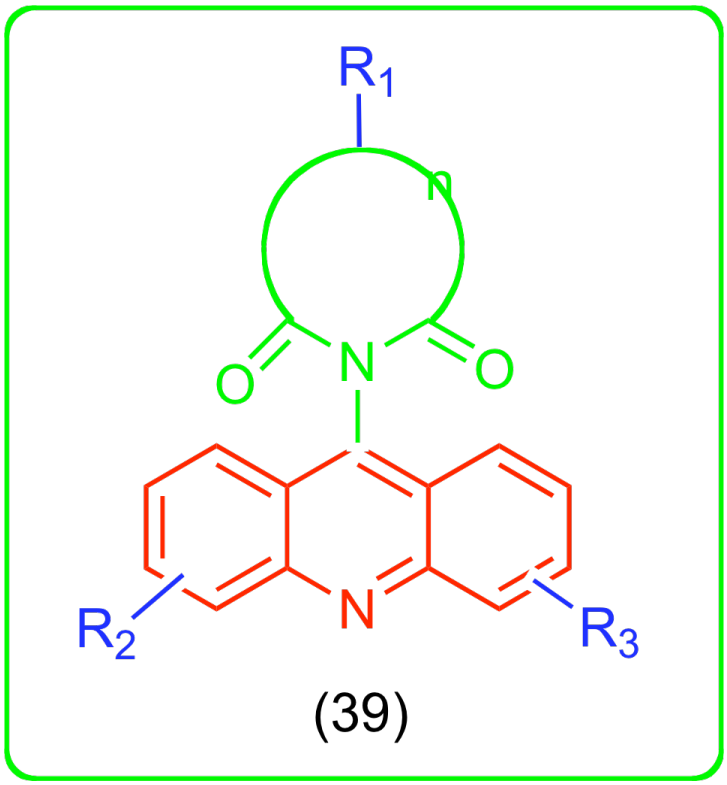


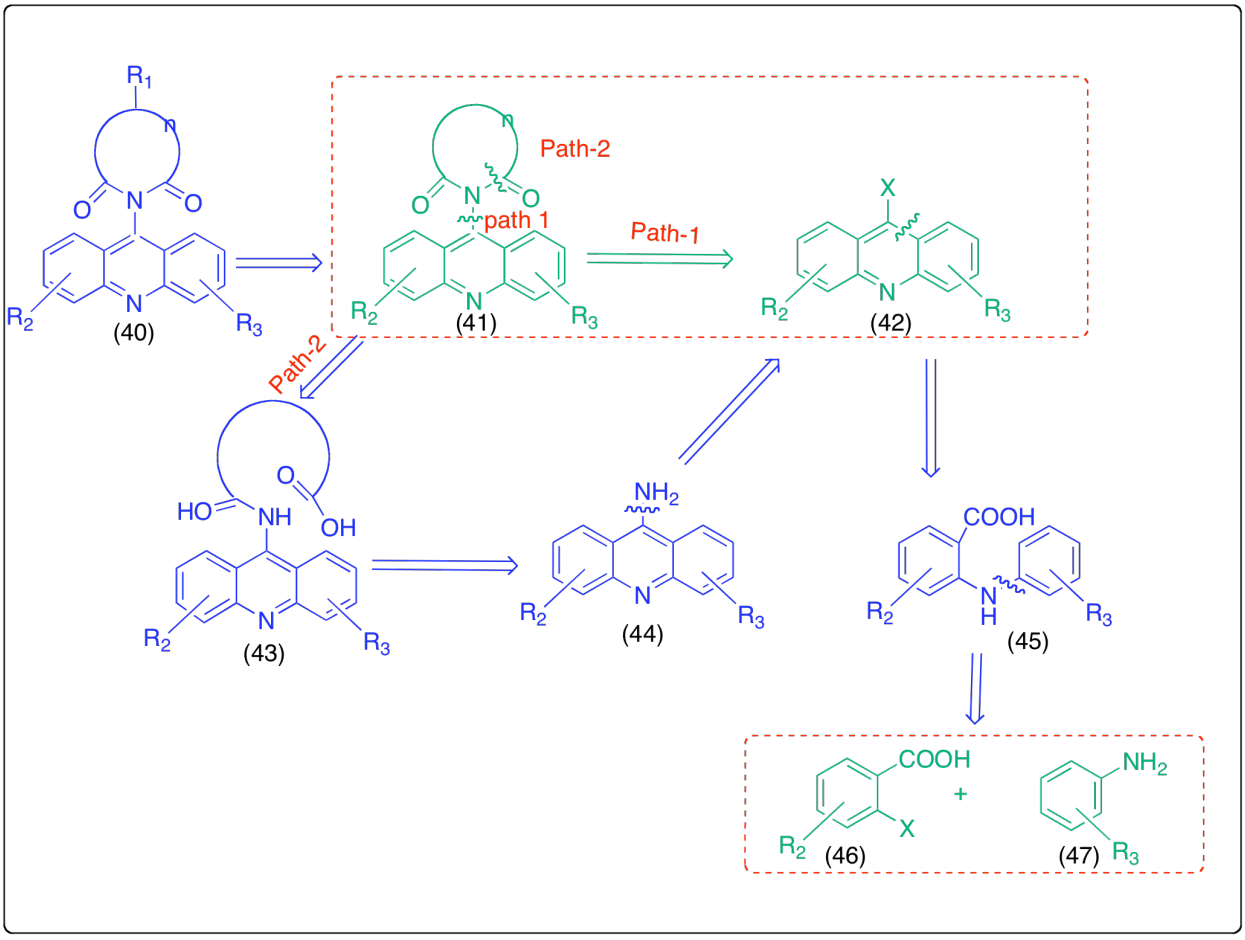
Figure 2.15 General structure of the proposed target molecule

We started with a basic acridine moiety and introduced a cyclic imide moiety at 9th position as a linker. Finally we were interested in introducing different substituents on acridine ring and as well as on cyclic imide moiety. We particularly interested in substituents like amino acids, small

peptide chains, because of bringing our molecule close to physiological conditions. Nara et al reported a few similar compounds as fluorometrical reagent for thiol compounds [20].

The retro synthetic analysis of the target molecule shown in the scheme

2.2.2 Retro synthesis of the target molecule:



Scheme 2.2 Retro synthesis of the target molecule

Based on the general structure, we proposed a retrosynthesis consists of two paths.

The final compound (40) can be synthesized by substitution on N-acridyl cyclic imide moiety (41). However, N-acridyl cyclic imide (41) can be synthesized in two paths.

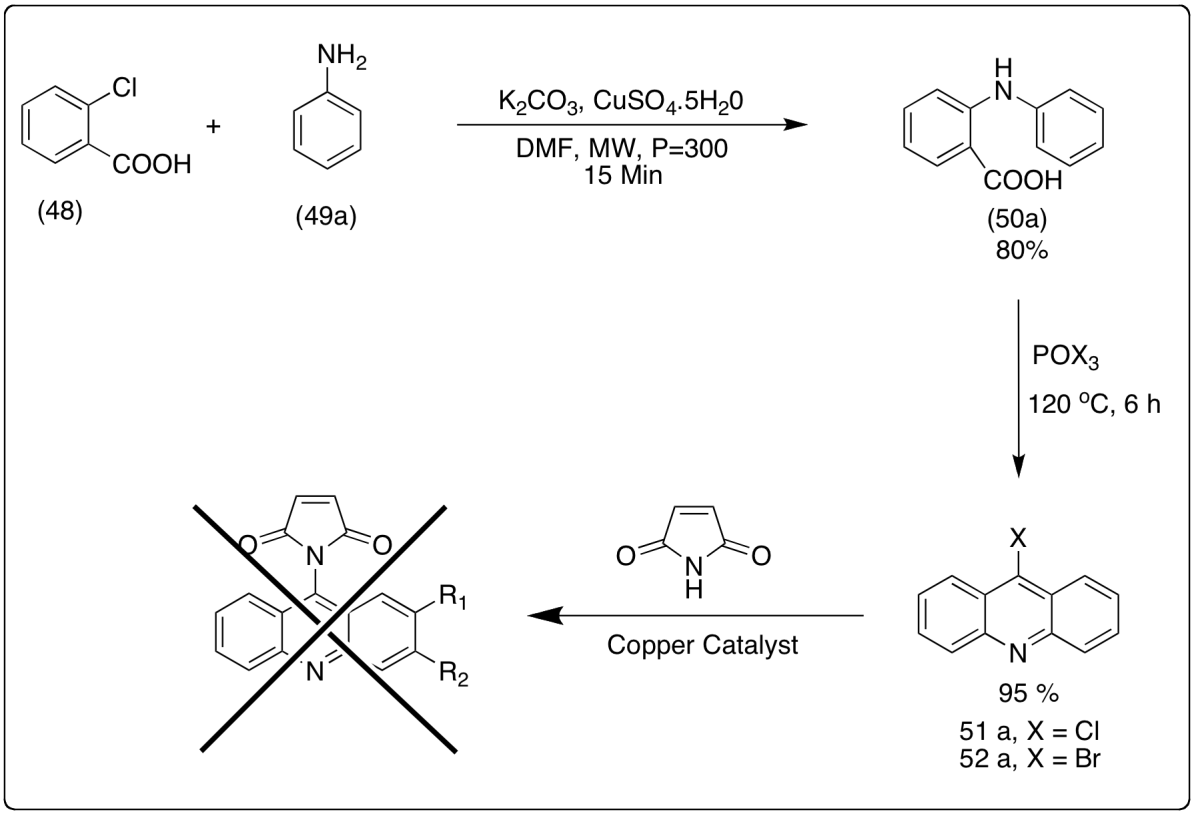
Path-1: In path-1, compound 41 can be obtained directly by coupling of 9-haloacridine with corresponding cyclic imide.

Path-2: In path-2, first 9-haloacridine (42) is converted to 9-aminoacridine (44) by nucleophilic substitution, and later on 9- aminoacridine is coupled with corresponding cyclic anhydride resulted to get compound 43 and this N-acridyl cyclic acid can be closed to get the compound 41. This path required, extra 2 steps then path-1.

9-haloacridine (42), will be synthesized by ring closing and aromatization of N-phenyl anthranilic acid derivatives (45). And finally N-phenyl anthranilic acids can be synthesized by coupling of 2-halobenzoic acid derivatives (46) with aniline derivatives (47).

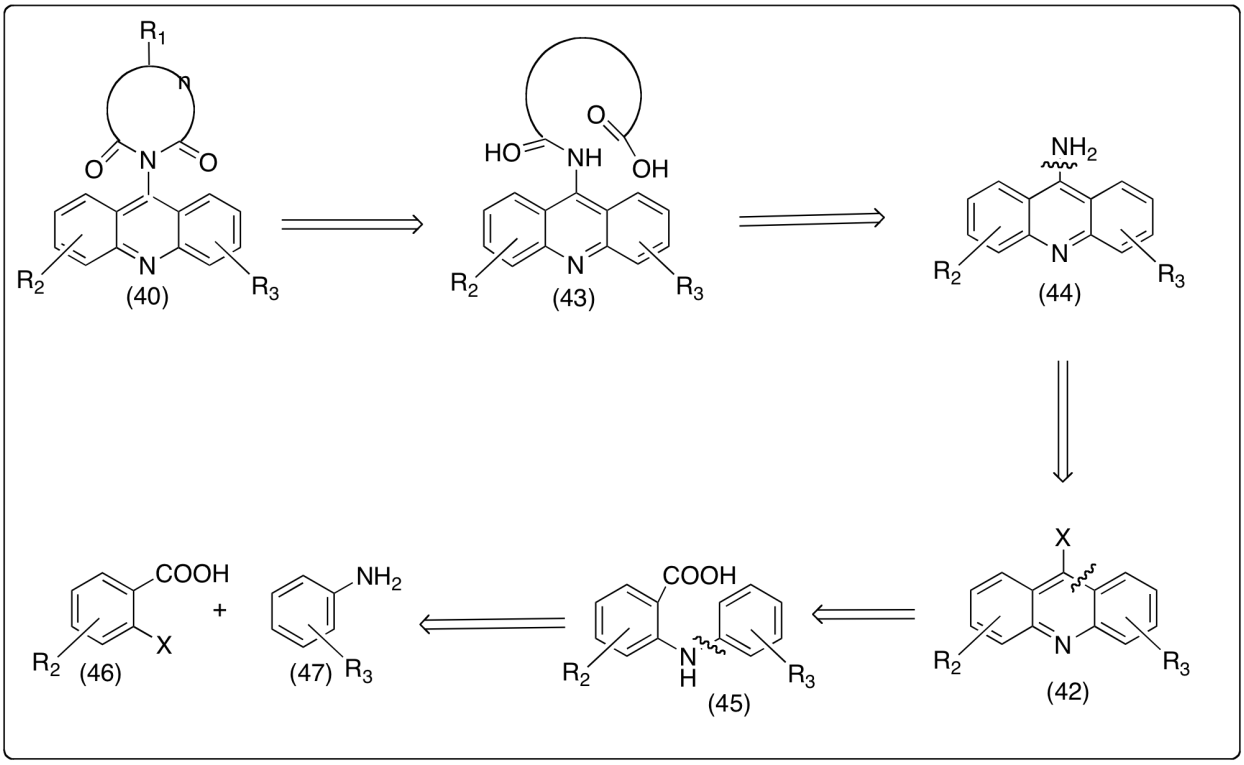
I started working on synthesizing the acridine derivatives by using path-1 (Scheme: 2.3):

Working with Path-1:



Scheme 2.3 Synthetic scheme for acridine derivatives by using path-1

The coupling of 2-chlorobenzoic acid with (48) with aniline (49) took place in the microwave and completes in 15 min to get the N-phenyl anthranilic acid (50a) with a percentage yield of 80. The N-phenyl anthranilic acid is refluxed in phosphorus oxy halides about 6h to get the 9-halo acridine. By using this, we synthesized 9-bromo and 9-chloro acridine in 95% yield. Finally, when I tried to couple 9- halo acridine with maleimide by using various copper catalysts, I ended up with no reaction. We were not interested in any Pd/Pt catalysts because of the problems associated with these catalysts. Because of not getting success with path-1, I changed the scheme to path-2. So the final retro synthesis analysis was shown in scheme 2.4.

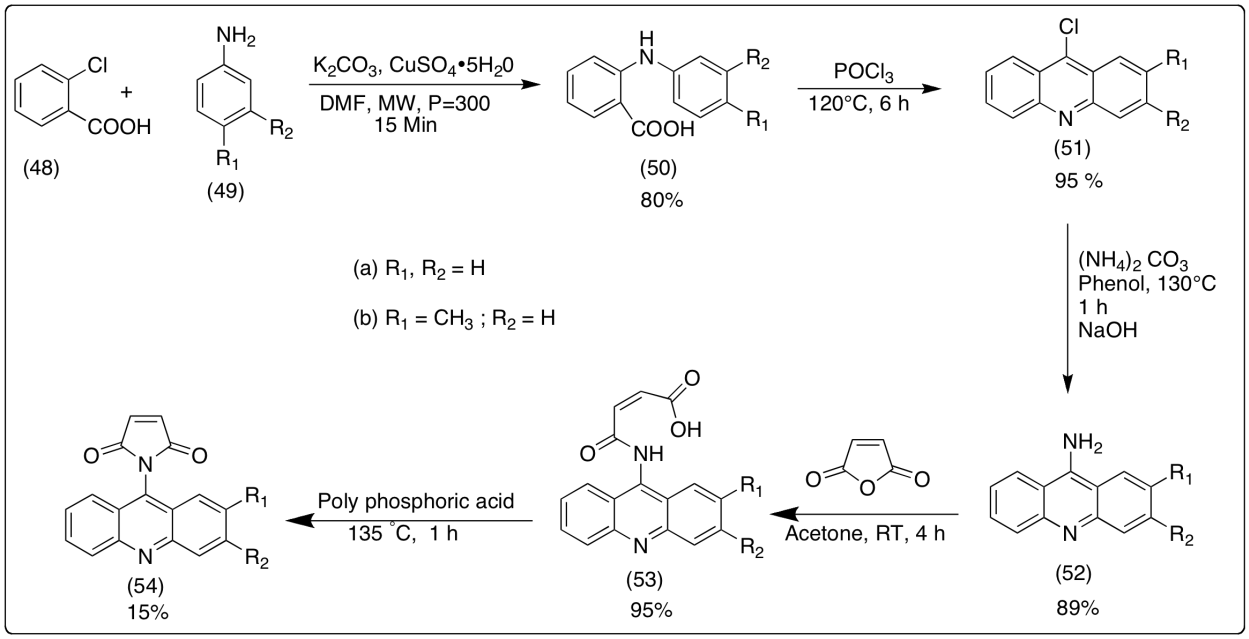


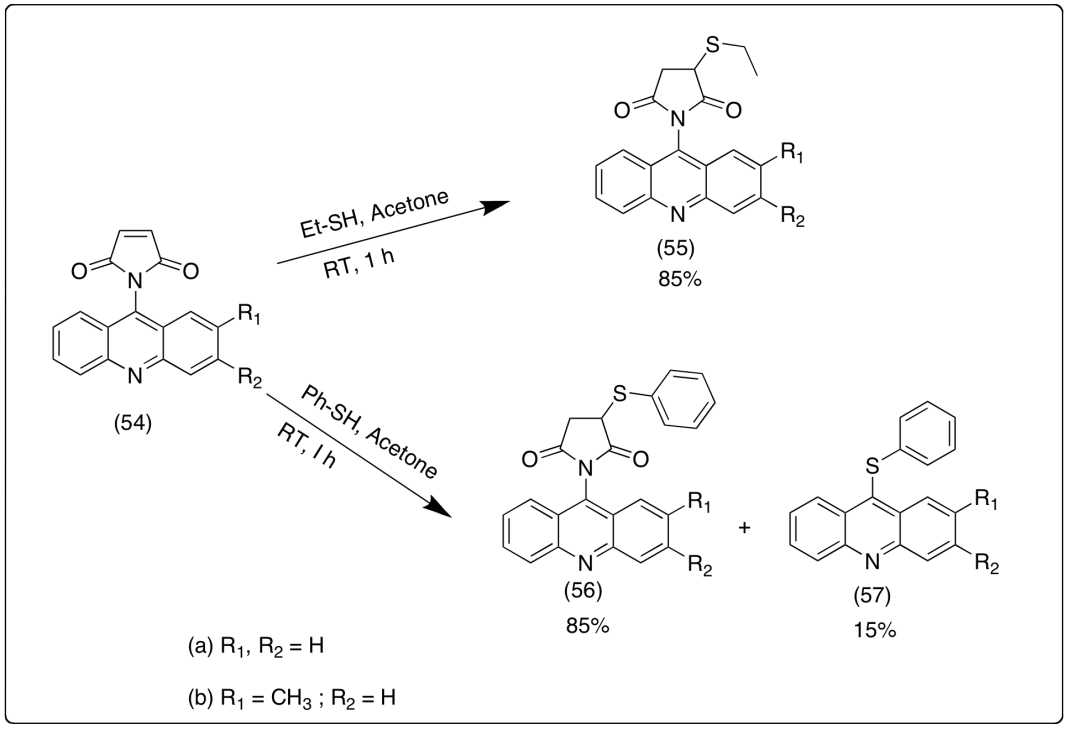
Scheme 2.4 Successful retro synthetic analysis of the target molecule

I started synthesizing the final molecules by using the scheme 2.5

2.3 Synthesis of Acridine derivatives:

2.3.1 Synthesis of N-acridyl maleimide (NAM) derivatives:





Scheme 2.5 Synthesis of N-acridyl maleimide (NAM) derivatives

N-phenyl anthranilic acids (50) were prepared by coupling of 2-chlorobenzoic acid with substituted anilines. The coupling reaction takes place in the microwave at a power of 300, the reaction completes in 15 min. N-phenyl anthranilic acids (50) will undergo ring closing and aromatization under refluxing conditions in $POCl_3$ to produce 9-chloroacridines (51), and we can convert them to 9-amino acridines by nucleophilic substitution of Cl by ammonium carbonate. After that, 9-aminoacridines (51) were coupled with maleic anhydride to get the N-acridyl maleic acid derivatives (53). The final molecule N-acridyl maleimide (NAM) (54) is obtained by ring closing of NAM-open (53) by using polyphosphoric acid.

Treating with thio alcohols in acetone under the room temperature conditions, NAM was further functionalized as we can see in the scheme.

By using these two schemes, I synthesized the following NAM based molecules.

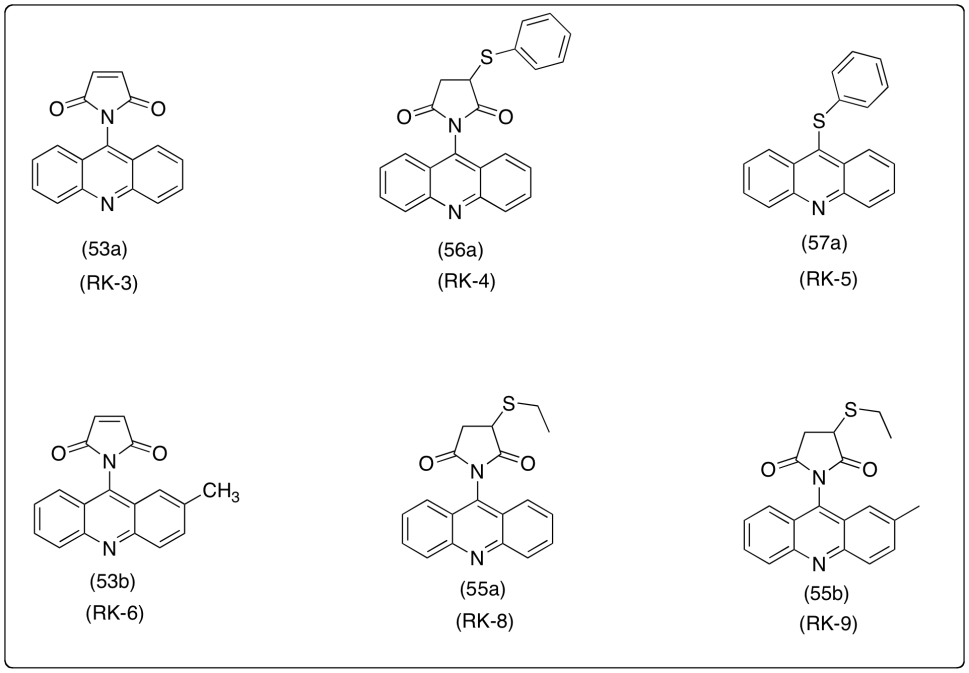
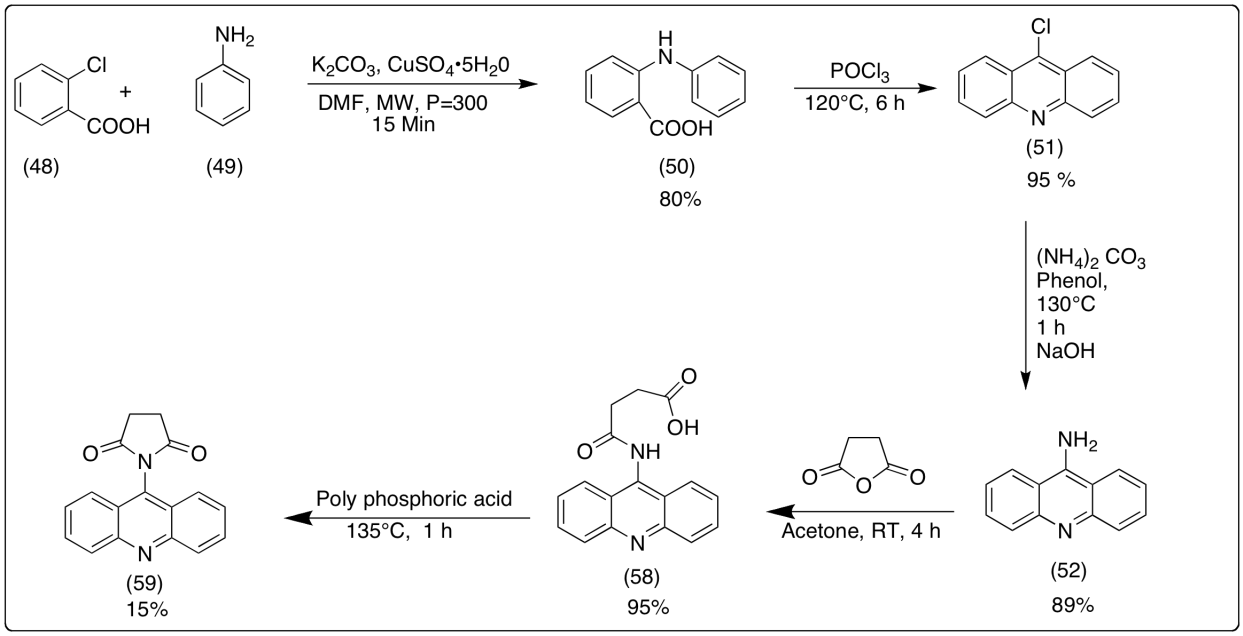


Figure 2.16 Synthesized N-acridyl maleimide (NAM) derivatives

2.3.2 Synthesis of N-acridyl succinimide (NAS) derivatives:

After synthesizing the NAM derivatives, by using the same schematic procedure I also synthesized the N-acridyl succinimide derivatives (NAS) (59). The only difference is, N-acridyl succinic acid (58) was obtained by coupling of 9-aminoacridine derivatives (52) with succinic anhydride.



Scheme 2.6 Synthesis of N-acridyl succinimide (NAS) derivatives

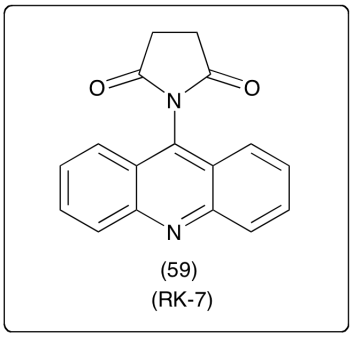
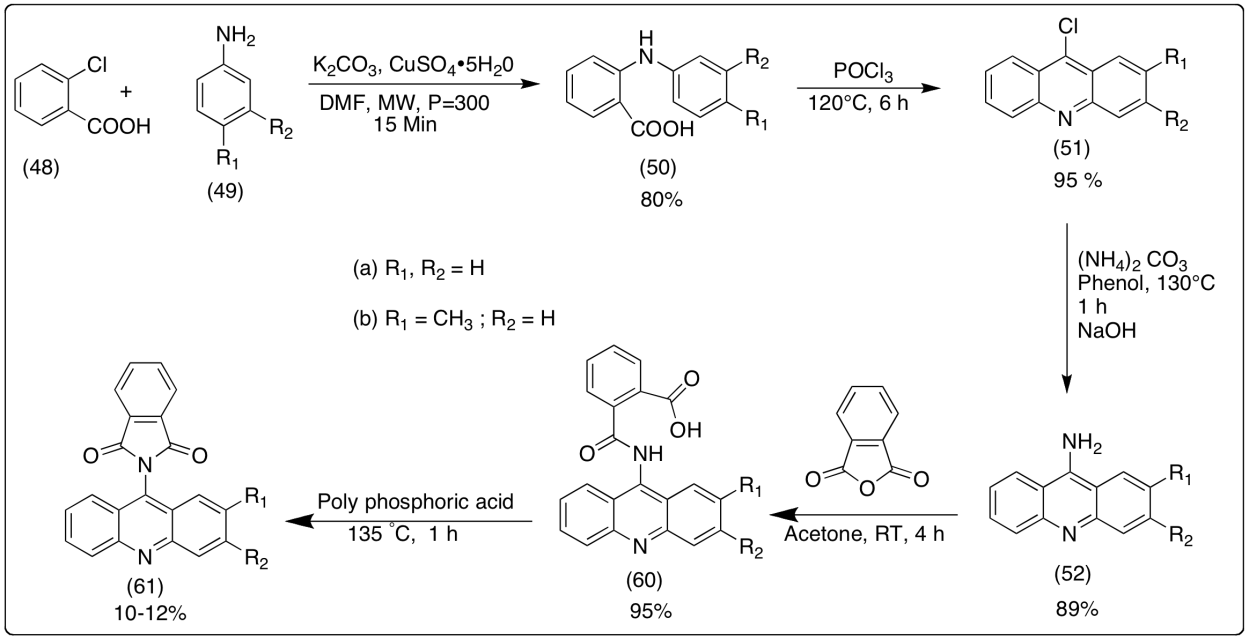


Figure 2.17 Synthesized N-acridyl succinimide (NAS) derivative

2.3.3 Synthesis of N-acridyl phthalimide (NAP) derivatives:

Finally, we synthesized the N-acridyl phthalimide derivatives (61a, 61b) by coupling the 9-amino acridine derivatives (52) with phthallic anhydride and followed by ring closing with poly phosphoric acid.



Scheme 2.7 Synthesis of N-acridyl phthalimide (NAP) derivatives

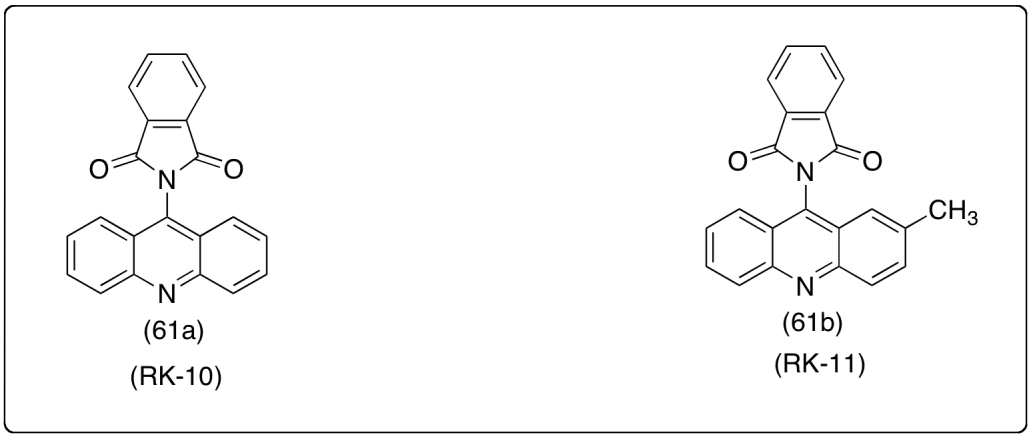
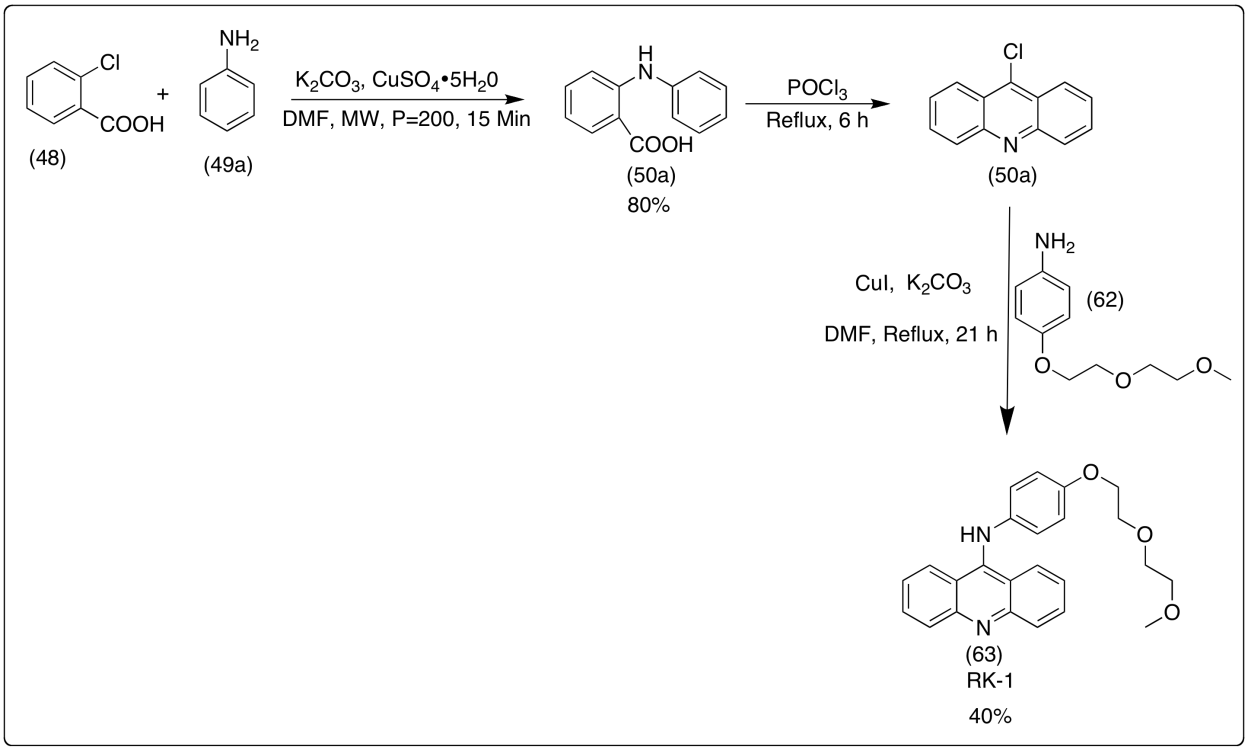


Figure 2.18 Synthesized N-acridyl phthalimide (NAP) derivatives

2.3.4 Synthesis of RK-1:

Along with the NAM, NAS and NAP derivatives I also synthesized N-aryl acridine derivatives. RK-1 (63) was synthesized by coupling of 9-chloroacridine (50a) with 4-(2-(2-methoxyethoxy)ethoxy)aniline. The coupling was catalyzed by CuI, and completes in 21 h of stirring under the refluxing condition in DMF.

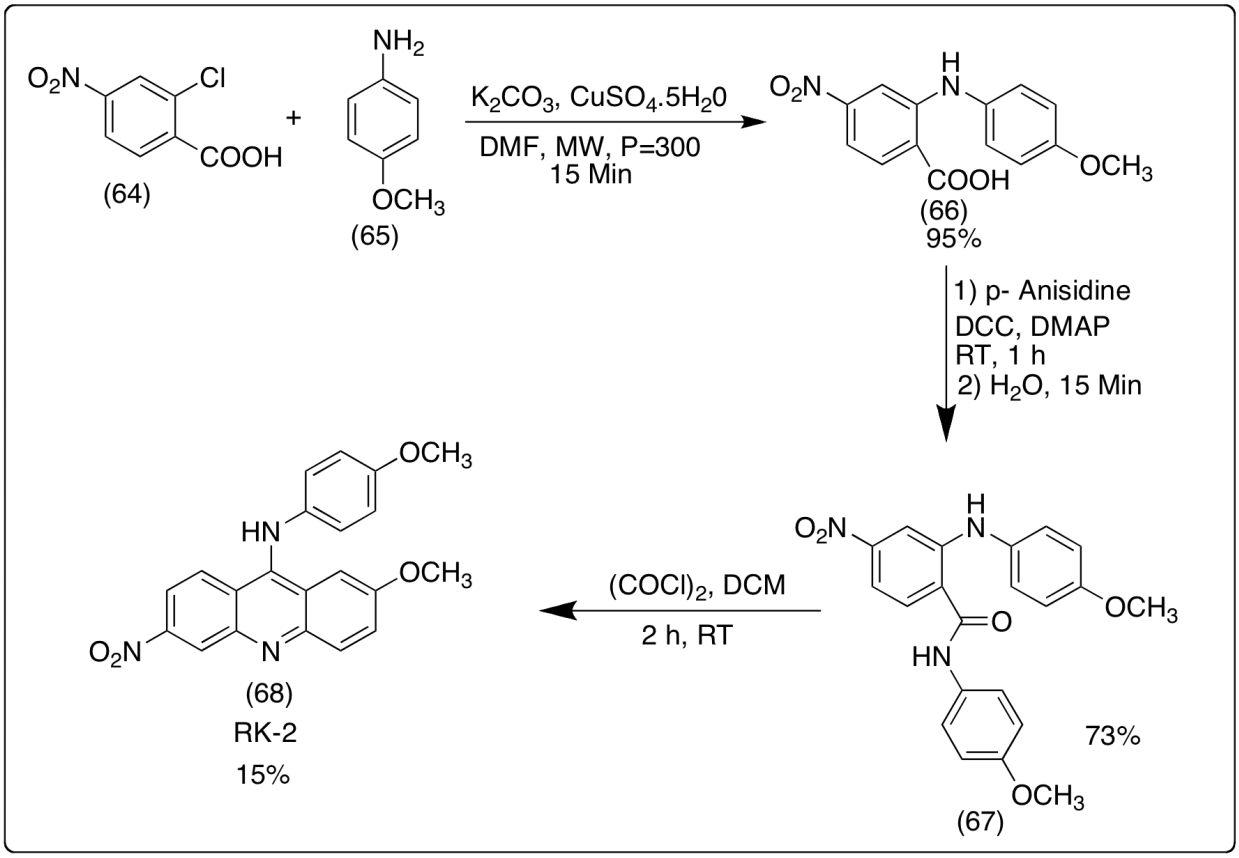


Scheme 2.8 Synthesis of RK-1

2.3.5 Synthesis of RK-2:

RK-2 was synthesized as shown in the scheme 2.8. Compound 66 was obtained by coupling of 2-chloro-4-nitrobenzoic acid (64) with *p*-anisidine (65). The N-phenyl anthranilic acid derivative (66) was coupled with *p*-anisidine in the presence of DCC to form the corresponding amide (67).

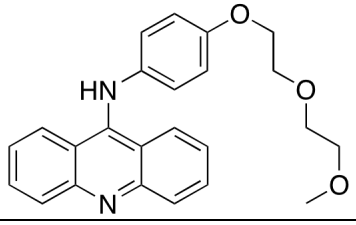
Finally, ring closing and aromatization takes place in oxalyl chloride solution to get the final compound RK-2 (68).

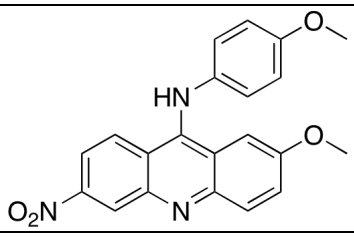
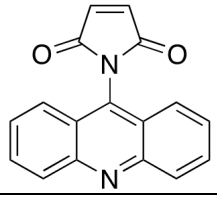
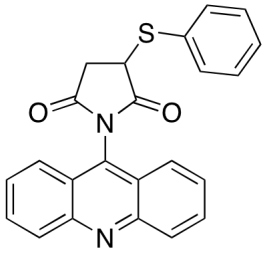
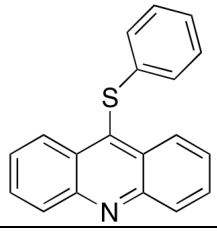
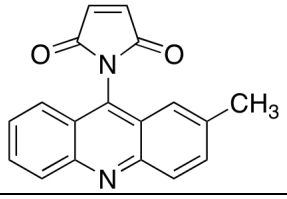
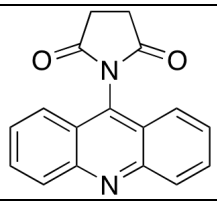
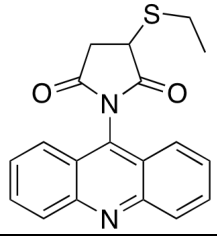


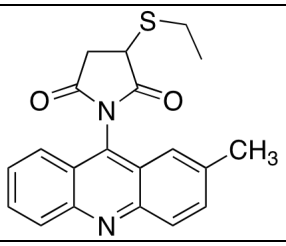
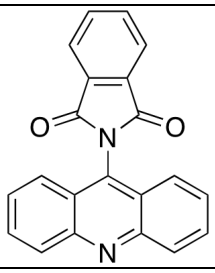
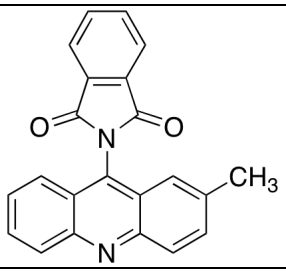
Scheme 2.9 Synthesis of RK-2

2.3.6 Synthesized acridine derivatives and their Log p values

Table 2.2 Synthesized acridine derivatives and their Log p values

NAME	STRUCTURE	MOLECULAR WEIGHT	LOG P
RK-1		**388.46**	**4.57**

RK-2		**375.38**	**5.13**
RK-3		**274.27**	**2.65**
RK-4		**371.43**	**4.46**
RK-5		**287.36**	**5.64**
RK-6		**288.30**	**3.13**
RK-7		**276.29**	**2.37**
RK-8		**336.41**	**3.14**

RK-9		**350.43**	**3.62**
RK-10		**324.33**	**4.16**
RK-11		**338.36**	**4.64**

2.4 Anti cancer assays of synthesized acridine based molecules:

After synthesizing a library of acridine derivatives, we tested them on various cancer cell lines. We conducted different types assays like MTT and STAT.

2.4.1 MTT Assays:

MTT assay− 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Aldrich) assay was performed according to manufacturer's protocol. The cancer cells and control cells cultured in 96 well plates for different time intervals were incubated with MTT reagent (100µg/well) for required time at 37°C. At the end of incubation, 100 µl of MTT solvent (4mM HCl, 0.1% NP-40 in isopropanol) was added to each well and was mixed well on a shaker for 15 min. Absorbance was recorded in a calorimeter at 590 nm (Spectra max 190, Molecular

Devices). Average values from at least three independent experiments were used to analyze the data.

2.4.1(a) MTT Assays of acridine derivatives on 786-0 cell line:

Cell line 786-0 (CRL-1932): Primary clear cell renal adenocarcinoma in a 58-yr-old male with multiple lung metastases.

1 μ. Mol:

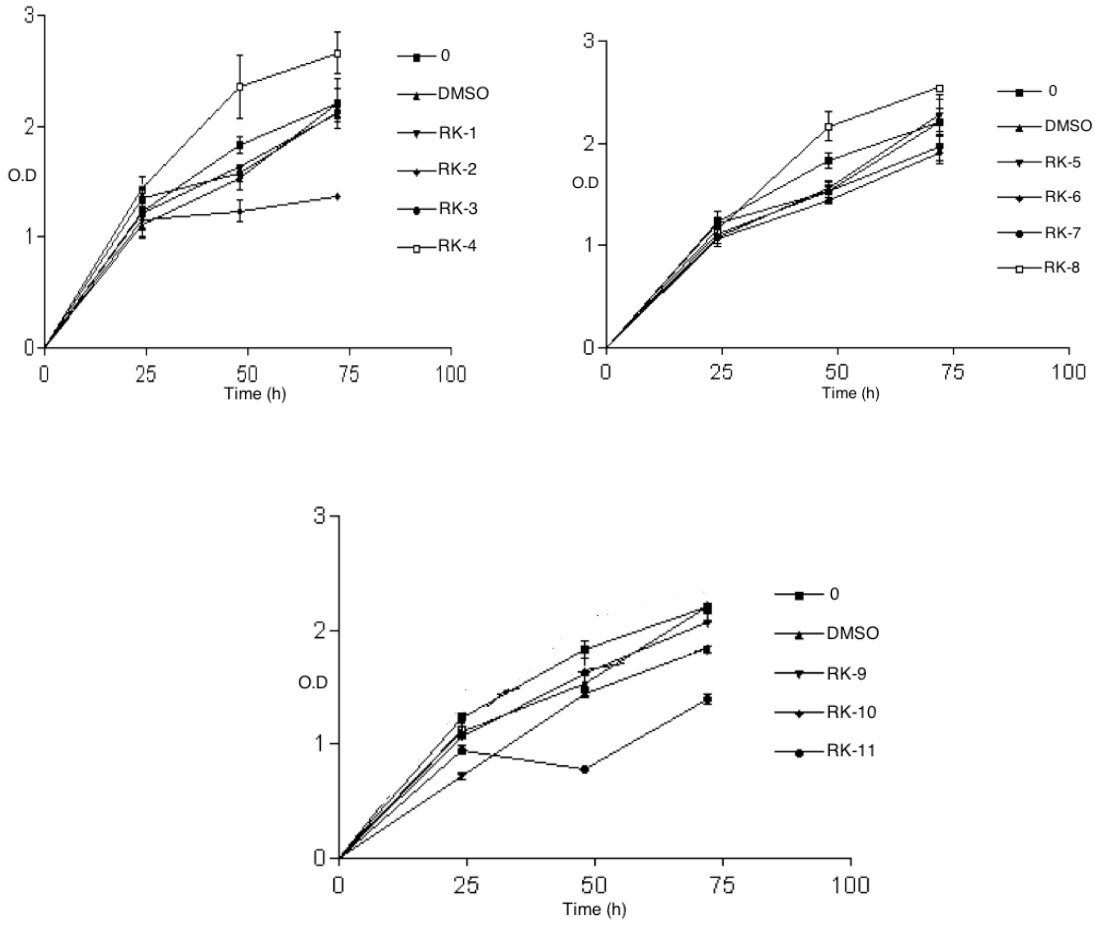


Figure 2.19 MTT assays of synthesized acridine derivatives on 786-0 cell lines at 1 μ. Mol concentration

10 µ. Mol:

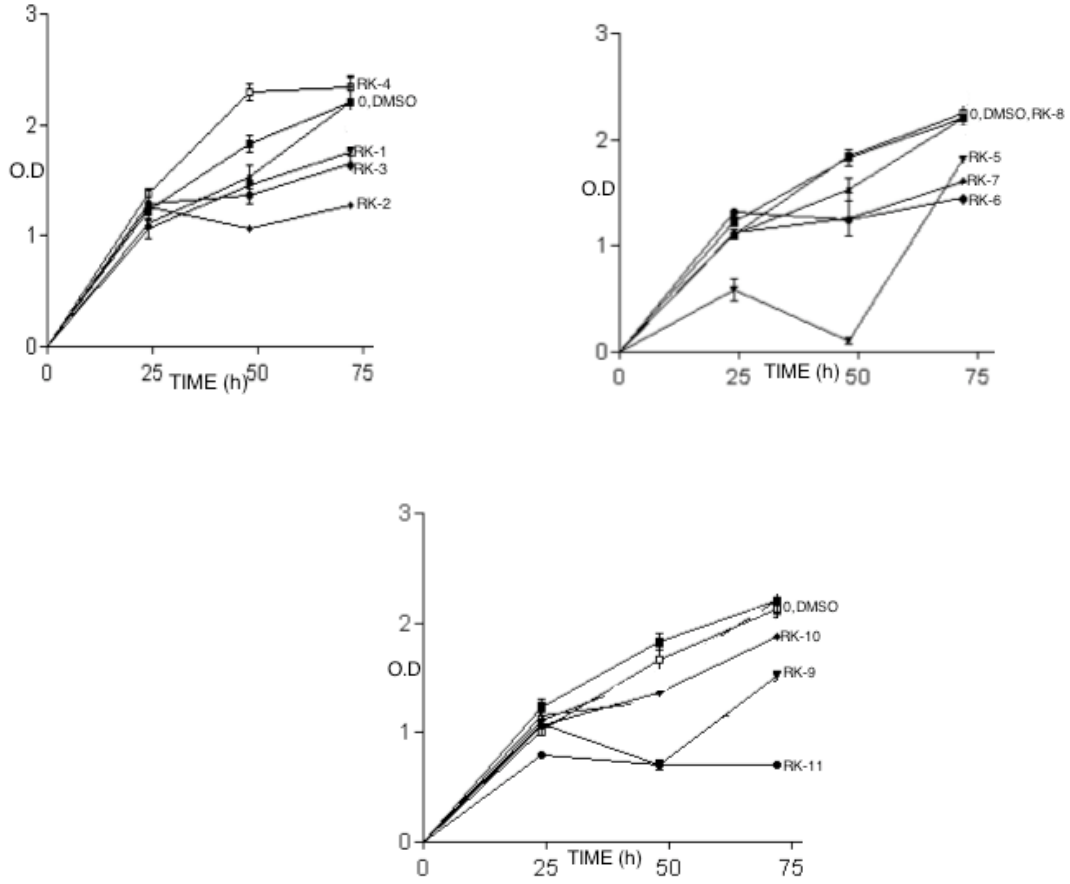
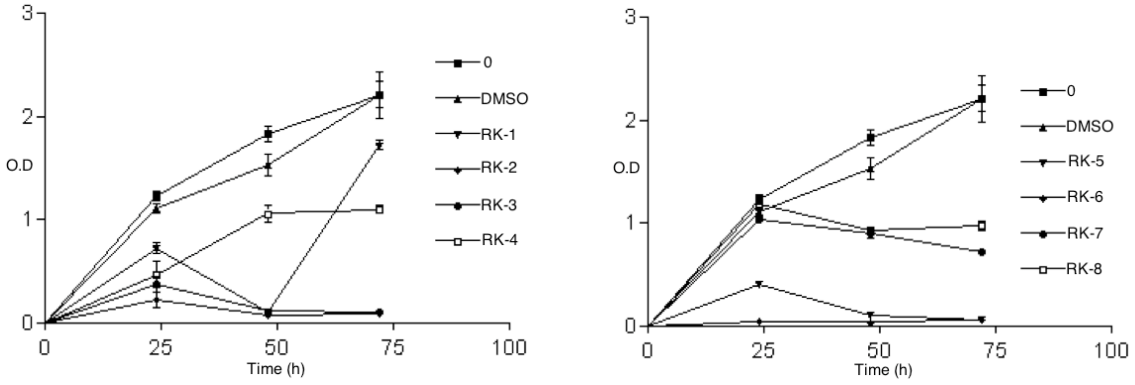


Figure 2.20 MTT assays of synthesized acridine derivatives on 786-0 cell lines at 10 µ. Mol concentration

100 µ. Mol:



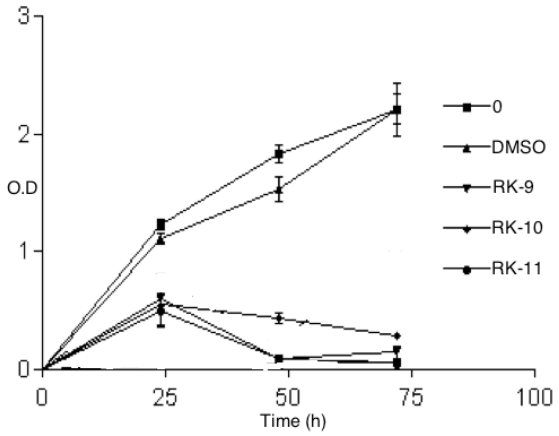
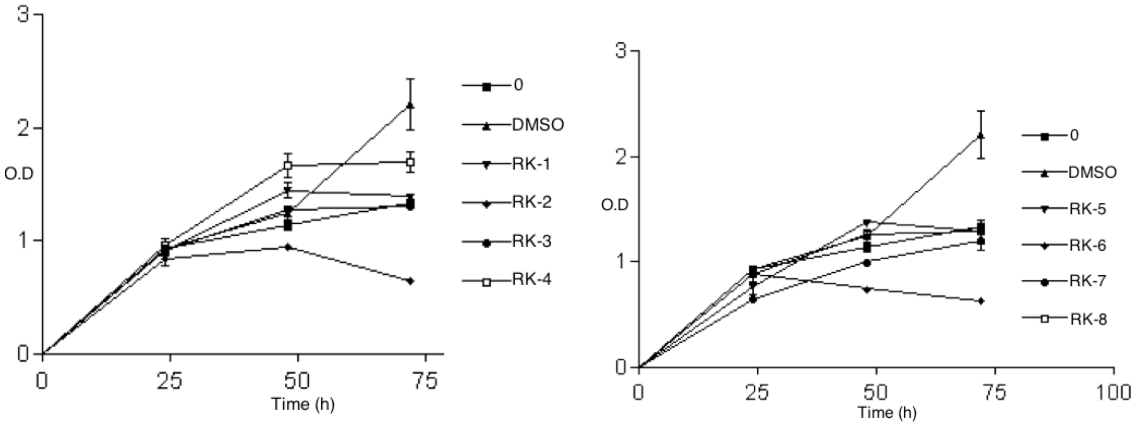


Figure 2.21 MTT assays of synthesized acridine derivatives on 786-0 cell lines at 100 μ. Mol concentration

2.4.1(a) MTT Assays of acridine derivatives on crl cell line:

crl cell line : Derived from the female renal adeno carcinoma

1 μ. Mol:



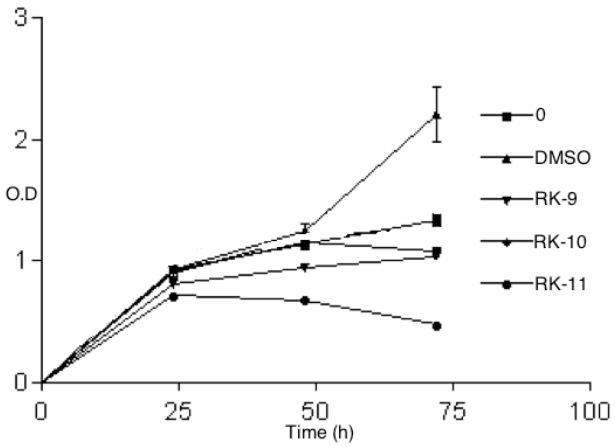
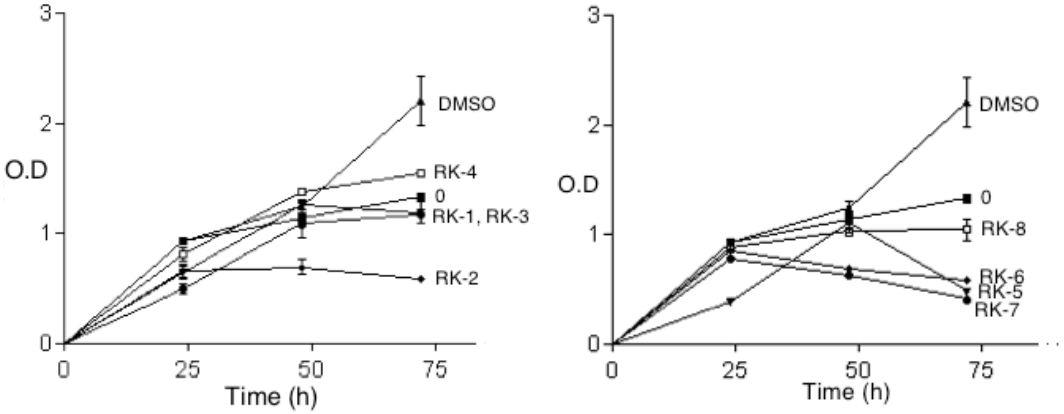


Figure 2.22 MTT assays of synthesized acridine derivatives on crl cell lines at 1 μ. Mol concentration

10 μ. Mol:



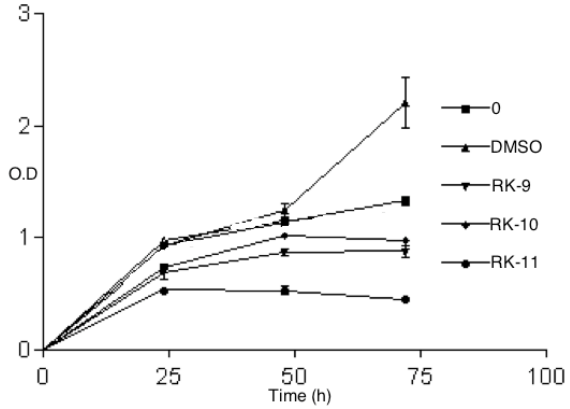


Figure 2.23 MTT assays of synthesized acridine derivatives on crl cell lines at 10 μ. Mol concentration

100 μ. Mol:

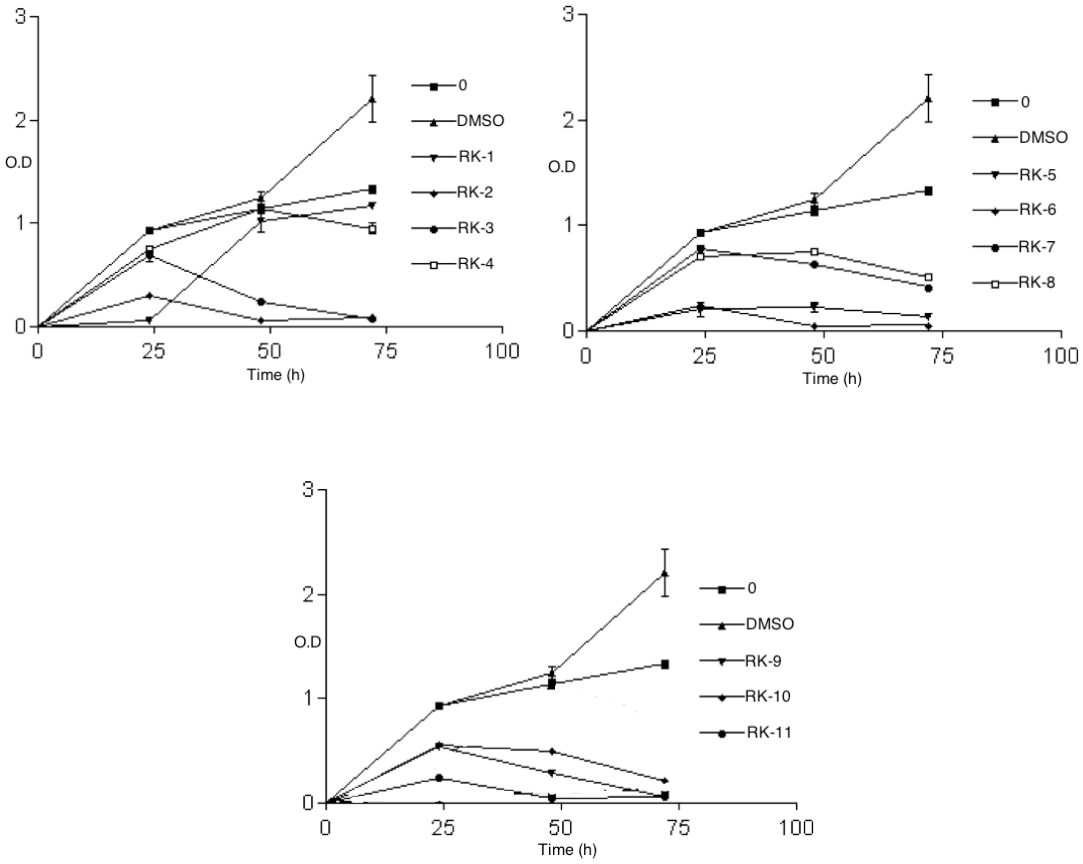


Figure 2.24 MTT assays of synthesized acridine derivatives on 786-0 cell lines at 100 μ. Mol concentration

2.4.1(c) MTT assays of acridine derivatives on RCC-4 cell line:

RCC-4: cells are derived from the primary tumor cells

1 µ. Mol:

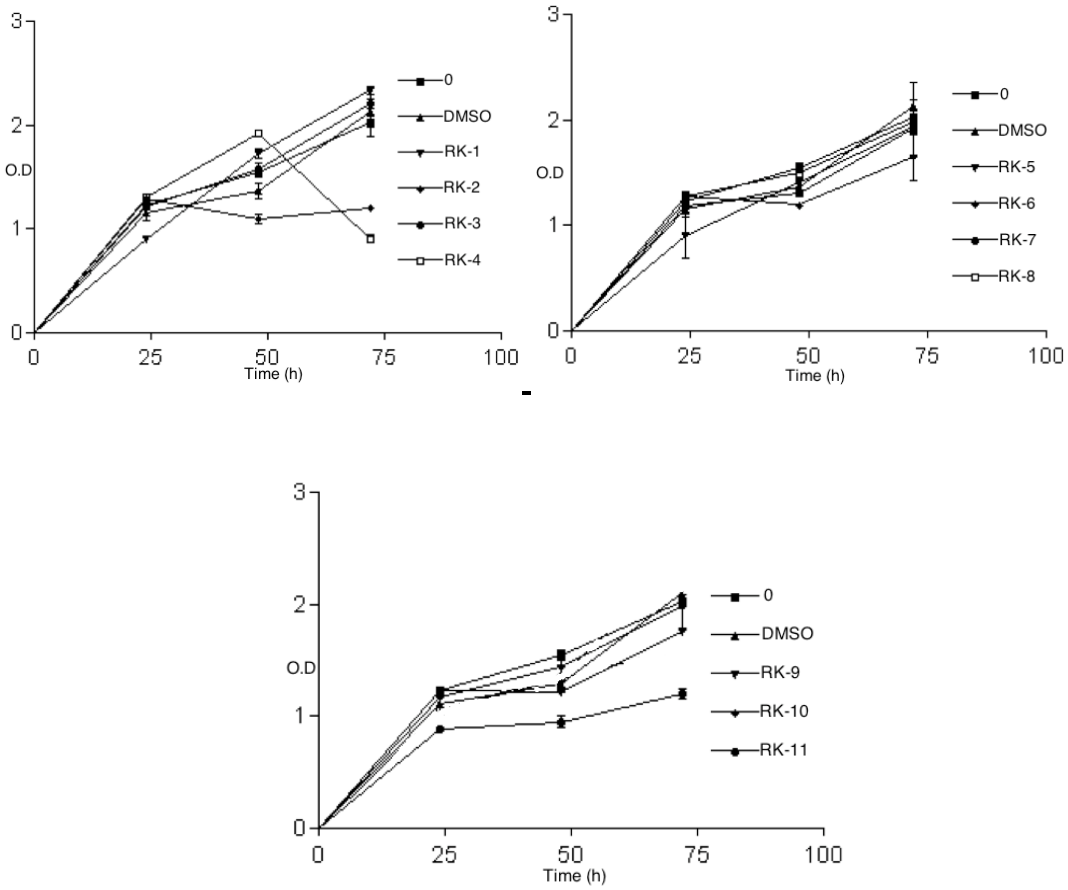
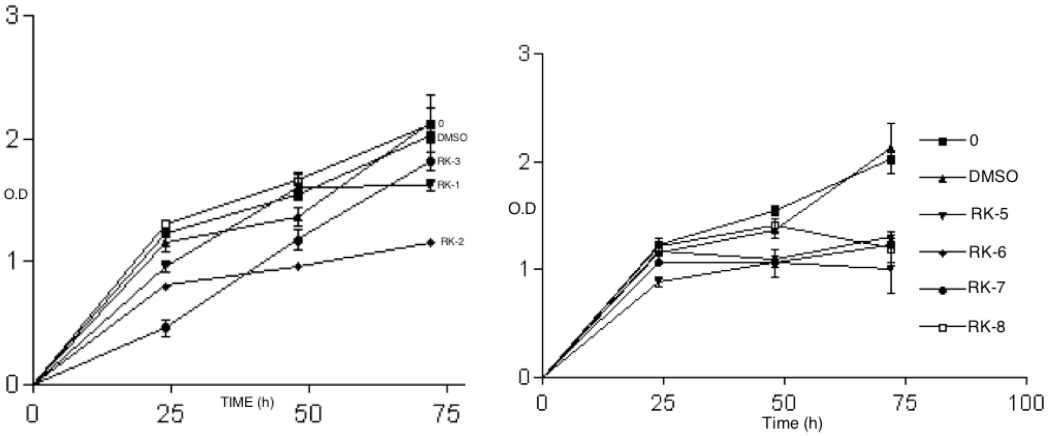


Figure 2.25 MTT assays of synthesized acridine derivatives on RCC-4 cell lines at 1 µ. Mol concentration

10 µ. Mol:



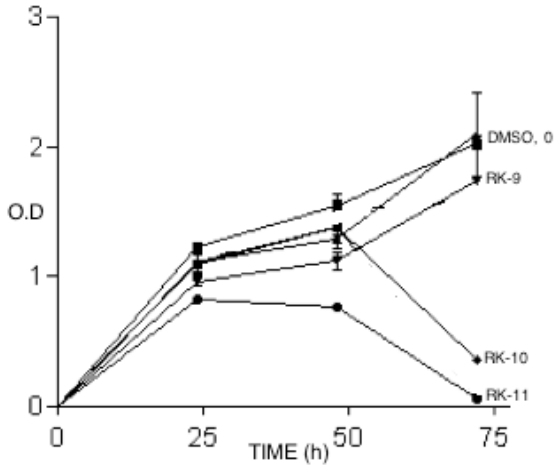
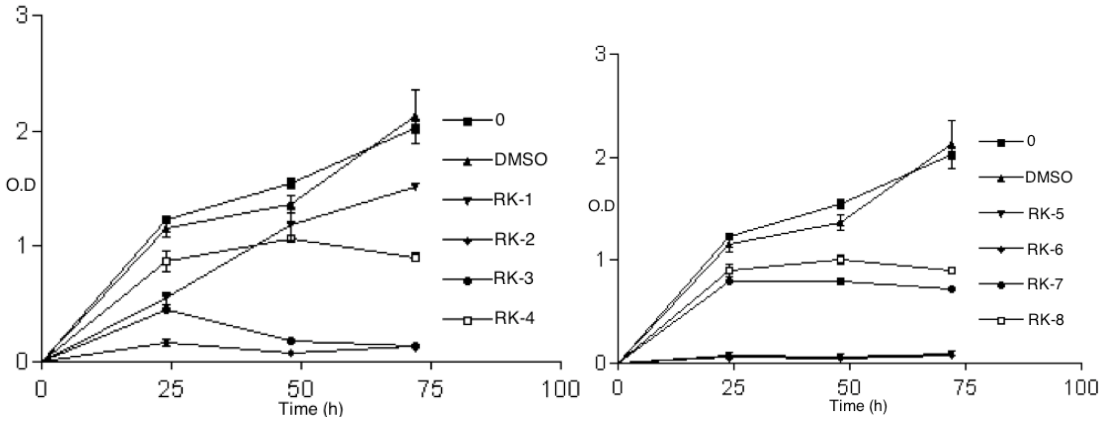


Figure 2.26 MTT assays of synthesized acridine derivatives on RCC-4 cell lines at 10 µ. Mol concentration

100 µ. Mol:



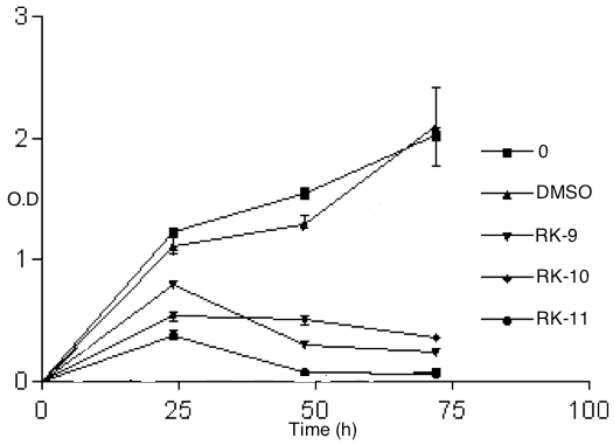


Figure 2.27 MTT assays of synthesized acridine derivatives on 786-0 cell lines at 100 μ. Mol concentration

2.4.2 STAT Analysis:

The Signal Transducer and Activator of Transcription or Signal Transduction And Transcription (STAT) family of proteins was first discovered in the 1990's as key proteins in cytokine signaling. They play important roles in numerous cellular processes including immune responses, cell growth and differentiation, cell survival and apoptosis, and oncogenesis.

There are seven mammalian STAT family members that have been identified: STAT1, STAT2 STAT3, STAT4, STAT5 (STAT5A and STAT5B) and STAT6.

2.4.2(a) STAT Proteins and Cancer:

Cancer associated inflammation is marked by the presence of specific inflammatory cells and inflammatory mediators, including cytokines and chemokines. Recent evidence suggests a crucial role for signal transducer and activator of transcription (STAT) family proteins – especially STAT3 in selectively inducing and maintaining a pro carcinogenic inflammatory microenvironment, both at the initiation of malignant transformation and during cancer progress. STAT3 is linked to inflammation-associated tumorigenesis that is initiated by genetic alterations

in malignant cells, as well as by many environmental factors, including chemical carcinogens, sunlight, infection, cigarette smoking and stress.

Aside from the tumor- promoting role of inflammation, many murine studies and clinical findings have underscored the importance of immune responses and inflammatory mediators – both naturally occurring and therapeutically induced- in suppressing tumorigenesis and tumor growth. STAT3 and, to some extent STAT5 and STAT6 are involved in inhibiting anti tumor immunity. However STAT1-STAT1 homodimers or STAT1-STAT2 heterodimers accumulate in the nucleus and regulate the expression of genes that promote growth arrest and apoptosis.

2.4.2(b) STAT Family and their Target Genes:

Table 2.3 STAT Family and their Target Genes

STAT Protein	Main Trget Genes
STAT1	T_H1- type immunostimulatory, and pro-apoptosis
STAT2	T_H1- type immunostimulatory, and pro-apoptosis
STAT3	T_H17-type anti-apoptosis, pro-proliferation, angiogenic and metastatic.
STAT4	T_H-1 type, especially IFNγ
STAT5A and STAT5B	Anti- apoptosis, pro- proliferation, and differentiation
STAT6	T_H2-type, and anti-apoptosis

IFN: Interferon, T_H: T helper

2.4.2(c) STAT Analysis of the Acridine Derivatives on Male Renal Adeno Carcinoma Cells:

Table 2.4 STAT analyses of the synthesized acridine derivatives on 786-0 cell line

786-0	STAT1	STAT2	STAT3	STAT5	STAT6
Control	48	29.5	719	73	24
RK-1	26	14	304	50	3.5
RK-2	35	31	63	54	21
RK-3	34	82	206	38	11
RK-5	24	19	73	53	16
RK-6	135.5	137	112.5	69	27
RK-9	38.5	44	65	31.5	10
RK-10	17	17	77.5	25	8
RK-11	11	10	28	19	9

2.4.2(d) STAT Analysis of the Acridine Derivatives on Male Primary Tumor Cells:

Table 2.5 STAT analyses of the acridine derivatives on RCC-4 cell line

RCC-4	STAT1	STAT2	STAT3	STAT5	STAT6
Control	26	18	583	40.5	9
RK-1	19	9.5	193	27.5	7
RK-2	17	14	55	29	3.5
RK-3	14	12	49	18	3.5

RK-5	16.5	15	105	51.5	5
RK-6	17	21	81	33	10
RK-9	24	30	99	42	6
RK-10	13	12	81	27	6.5
RK-11	28	46	70.5	58	14

2.5 Conclusions:

We have successfully synthesized a library of N-acridyl maleimide (NAM), N-acridyl succinimide (NAS) and N-acridyl phthalimide (NAP) derivatives. We also tested them on various cancer cell lines such as male renal adeno carcinoma (786-0), female renal adeno carcinoma (crl) and primary tumor cell lines (RCC-4) by using MTT assays. From the biological assays we got some ambiguous results. However by combining all the assays we can conclude that RK-3, RK-6, RK-10 and RK-11 have the better potency at 10 μ. Mol concentration. Even though most of the molecules showing better results at 100 μ. Mol concentration, we ruled out this concentration because of cytotoxicity of these molecules at higher concentrations. Along with MTT assays we also conducted the STAT assays and compared our MTT results with STAT assay results.

2.6 Experimental Section:

N-phenyl anthranilic acids (50):

A mixture of 2-chlorobenzoicacid (**48**) (11.7 gm, 0.0740 m) and corresponding aniline derivative (**49**) (0.0830 m) in 100 ml of DMF was brought to reflux in a microwave at a power of 300 and continued stirring about 15 min, cooled and filtered the precipitate. The product was extracted by DCM and washing the organic layer with water. Combined organic layers were dried under sodium sulfate after that a quick flash column with DCM results in getting the N-phenyl anthranilic acid derivatives in mentioned isolated yields.

9-chloroacridine Derivatives (51):

A mixture of N- phenyl anthranilic acid derivatives (**50**) (5.0 mmol) in 10 ml of phosphorous oxy chloride is stirred about 6 h at a temperature of 120^0C, cooled and poured in a vigorous stirring solution of an ice cold mixture of chloroform and ammonia in water (Maintain the pH of solution around 7-8). Washed the organic layer with cold water, and dried under sodium sulfate. Combined organic layers were dried under reduced pressure to get the crude 9-chloroacrdine (**51**) derivatives, and used them in the next step without purification.

9-Aminoacridine Derivatives (52):

A solution of 9-chloroacridine derivatives (**51**) (11.0 mmol) in 15 ml of phenol was stirred at a temperature of 70°C. To this stirring solution (18.0 mmol) of ammonium carbonate was added, immediately we can observe the raise in temperature to 130-135°C, and continued the stirring at this temperature about 1 h, cooled to room temperature and poured in a 50 ml of ice cold acetone

and kept about 1 h resulting a precipitate of 9-aminoacridine hydrochloride. Filter the precipitate and washed the precipitate with acetone and dried. The hydrochloride salt was dissolved in hot water and charged with sodium hydroxide solution in water resulting a precipitate of free 9-aminoacridine derivatives, filter the precipitate and washed with boiling water and dried to get the pure 9-aminoacridine derivatives.

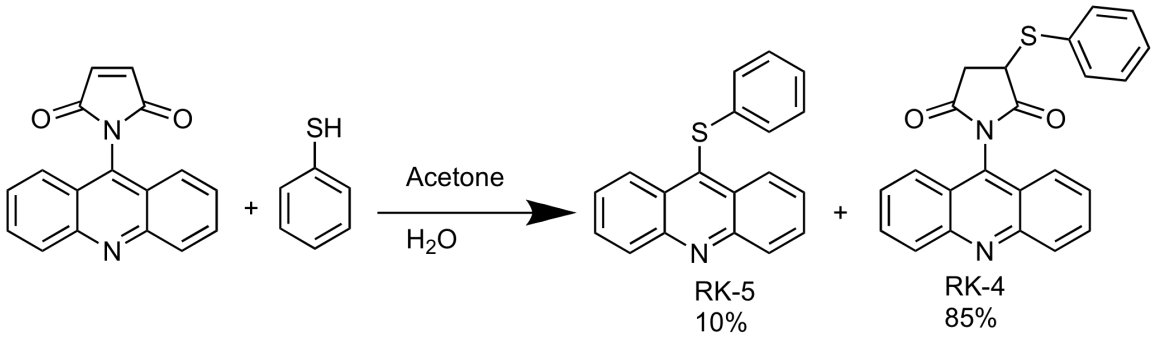
N-(9-Acridinyl)Maleamic Acid Derivatives (53):

To a stirring solution of 9-aminoacridine deribative (**52**) (3.0 mmol) in 40 ml of acetone, (15.0 mmol) of maleic anhydride in 8 ml of acetone was added and the resulting solution stirred at room temperature about 4 h resulting an yellow colored precipitate, filtered the precipitate and washed several times with acetone to get the pure product of N-(9-acridinyl)maleamic acid derivatives with corresponding yields.

N- (9-Acridinyl)Maleiimide Derivatives (54);

(2mmol) of N-(9-acridinyl)maleamic acid (**53**) was added to 8.0 g of polyphosphoricacid and the resulting slurry was stirred at a temperature of 135°C about 1 h, cooled to the room temperature and poured on ice resulting the yellow colored precipitate. Filter the precipitate and neutralize with sodium bicarbonate powder resulting a yellow colored precipitate, filtered it and washed with water and dried under vaccume resulting the pure N-(9-acridyl)maleimide derivatives (**54**) with reported yields.

[1-(acridin-9-yl)-3-(phenylthio)pyrrolidine-2,5-dione (RK-4) (56a):



To a solution of NAM (RK-3) (**53a**) (274 mg, 1.00 mmol) in 120 ml of acetone, (0.12 ml, 1.1 mmol) of thiophenol and 1 ml of water were added. The mixture was stirred at room temperature about 30 min, and then acetone was evaporated at 30°C under reduced pressure, the resulting precipitate was filtered washed with water and extracted with DCM and upon purification by using column chromatography with DCM to get the 327 mg of pure 1-(acridin-9-yl)-3-(phenylthio)pyrrolidine-2,5-dione (RK-4) (**56a**) with a 85% yield.

9-(phenylthio)acridine (RK-5) (57a):

To a solution of NAM (RK-3) (**53a**) (274 mg, 1.00 mmol) in 120 ml of acetone, (0.12 ml, 1.1 mmol) of thiophenol and 1 ml of water were added. The mixture was stirred at room temperature about 30 min, and then acetone was evaporated at 30°C under reduced pressure, the resulting precipitate was filtered washed with water and extracted with DCM and upon purification by using column chromatography with 1%EtOAc+99% DCM to get the 28.7 mg of pure 9-(phenylthio)acridine (RK-5) (**57a**) with a 10% yield.

[1-(acridin-9-yl)-3-(ethylthio)pyrrolidine-2,5-dione (RK-8) (55a):

To a solution of NAM (RK-3) (**53a**) (274 mg, 1.00 mmol) in 120 ml of acetone, (0.08 ml, 1.1 mmol) of thioethanol and 1 ml of water were added. The mixture was stirred at room

temperature about 30 min, and then acetone was evaporated at 30°C under reduced pressure, the resulting precipitate was filtered and washed with water and dried to get the 306 mg of pure 1-(acridin-9-yl)-3-(ethylthio)pyrrolidine-2,5-dione (RK-8) (**55a**) with a 91% yield.

[3-(ethylthio)-1-(2-methylacridin-9-yl)pyrrolidine-2,5-dione (RK-9) (55b) :

To a solution of 2-Methyl NAM (RK-6) (**53b**) (288 mg, 1.00 mmol) in 120 ml of acetone, (0.08 ml, 1.1 mmol) of thioethanol and 1 ml of water were added. The mixture was stirred at room temperature about 30 min, and then acetone was evaporated at 30°C under reduced pressure, the resulting precipitate was filtered and washed with water and dried to get the 326 mg of pure 3-(ethylthio)-1-(2-methylacridin-9-yl)pyrrolidine-2,5-dione(RK-9) (**55b**) with a 93% yield.

N-(9-Acridinyl)succinic Acid (58):

To a stirring solution of 9-aminoacridine (**52**) (583 mg, 3.00 mmol) in 40ml of acetone, (15.0 mmol) of succinic anhydride in 8ml of acetone was added and the resulting solution stirred at room temperature about 4 hrs resulting an yellow colored precipitate. Filter the precipitate and washed several times with acetone to get the 821 mg of pure product of N- (9-acridinyl) succinic acid (**58**) with a percentage yield of 93%.

N- (9-Acridinyl)succinimide(NAS) (RK-7) (59):

N-(9-acridinyl) succinic acid (**58**) (2.0 mmol) was added to 8.0 gm of polyphosphoricacid and the resulting slurry was stirred at a temperature of 135°C about 1h, cooled to the room temperature and poured on ice resulting the yellow colored precipitate. Filter the precipitate and neutralize the elutent with sodium bicarbonate powder resulting a yellow colored precipitate,

filtered it and washed with water and dried under vaccume resulting the 82.9 mg of pure N-(9-acridyl)succinimide with isolated yield of 15%.

N-(9-Acridinyl)phthallic Acid (60):

To a stirring solution of 9-aminoacridine derivatives (**52**) (1.0 mmol) in 20 ml of acetone, (10.0 mmol) of phthallic anhydride in 10 ml of acetone was added and the whole solution stirred at room temperature about 4 h resulting an yellow colored precipitate. Filtered the precipitate and washed several times with acetone to get the pure product of N-(9-acridinyl) phthallic acid derivatives with corresponding yields .

N- (9-Acridinyl)phthalimide(NAP) (RK-10) (61a):

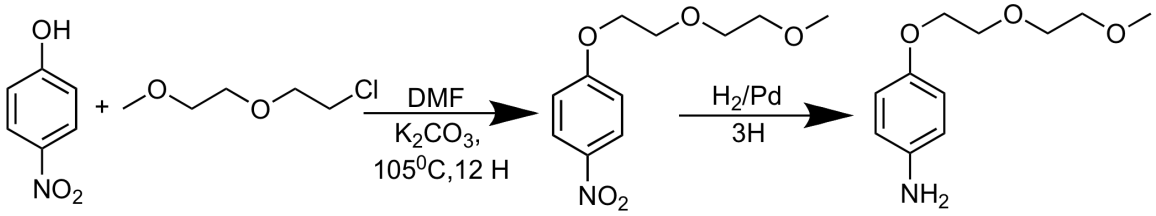
(2 mmol) of N-(9-acridinyl)phthallic acid (**60a**) was added to 8.0 g of polyphosphoricacid and the resulting slurry was stirred at a temperature of 135°C about 1 h, cooled to the room temperature and poured on ice resulting the yellow colored precipitate. Filter the precipitate and neutralize the elutent with sodium bicarbonate powder resulting a yellow colored precipitate, filtered it and washed with water and dried under vaccume resulting the 77.8 mg of pure N-(9-acridyl)phthalimide (RK-10) (**61a**) with isolated yield of 12%.

2-(2-methylacridin-9-yl)isoindoline-1,3-dione (RK-11) (61b):

2-methyl-N-(9-acridinyl)phthallic acid (2.0 mmol) (**60b**) was added to 8.0 gm of polyphosphoricacid and the resulting slurry was stirred at a temperature of 135°C about 1 h, cooled to the room temperature and poured on ice resulting the yellow colored precipitate. Filter the precipitate and neutralize the elutent with sodium bicarbonate powder resulting a yellow

colored precipitate, filtered it and washed with water and dried under vaccume resulting the 67.6 mg of pure 2-methyl-N-(9-acridyl)phthalimide (RK-11) (**61b**) with isolated yield of 10%.

4-(2-(2-methoxyethoxy)ethoxy)aniline (62):



4-nitro phenol (2085 mg, 15.00 mmol) and (2650 mg, 19.20 mmol) potassium carbonate were dissolved in 15 ml of DMF and the resulting mixture was brought to 105°C, to this stirring solution (2660 mg, 19.20 mmol) of (2-(2-chloroethoxy)ethoxy)methylium in 10 ml of DMF was added slowly about 10 min, upon completion the whole reactions mixture was stirred at 105°C about 12 h, cooled to the room temperature and extracted the organic layer with ether washed with 2M HCl and finally washed with 2M NaOH, combined organic layers dried under sodium sulfate and removed the solvent under reduced pressure to get the 3220 mg of 1-(2-(2-methoxyethoxy)ethoxy)-4-nitrobenzene with an isolated yield of 89%.

1-(2-(2-methoxyethoxy)ethoxy)-4-nitrobenzene is dissolved in minimum amount of methanol, to this added a pinch of Pd/C and the reaction mixture stirred under pressure in the H_2 environment about 3 h, after completion filtered the Pd/C, and evaporated the solvent under reduced pressure to yield the 2622 mg of 4-(2-(2-methoxyethoxy)ethoxy)aniline (**62**) with 93%.

N-(4-(2-(2-methoxyethoxy)ethoxy)phenyl)acridin-9-amine (RK-1) (63):

To a stirring solution **(**427 mg, 2.00 mmol) of 9-chloroacridine (**51a**) in 20 ml of DMF, (423 mg, 2.00 mmol) of 4-(2-(2-methoxyethoxy)ethoxy)aniline (**62**) and (278 mg, 2.00 mmol) of

potassium carbonate and (380 mg, 2.00 mmol) of copper (I) iodide were added and the resulting reaction mixture was brought to reflux about 21 h, after completion cool it down to the room temperature and filter the precipitate, the organic layer was extracted with the DCM and washed with water followed by 10% HCl solution. Combined organic layers were dried under sodium sulfate and done a column chromatography with 2% EtOAc + 98% DCM to give the 300 mg of pure product with a percentage yield of 40%.

N-(4-methoxyphenyl)-2-(4-methoxyphenylamino)-4-nitrobenzamide (67):

To a stirred solution of 2-(4-methoxyphenylamino)-4-nitrobenzoic acid (**66**) (576 mg, 2.00 mmol) in 20 ml of DCM, (246 mg, 2.00 mmol) of p-anisidine and (604 mg, 3.00 mmol) of DCC and a small amount of DMAP were added. The resulting reaction mixture stirred about 1 h at room temperature after that added couple of drops of water and stirred further more about 15 min, filtered of the precipitate and organic layer was extracted with the DCM and dried over sodium sulfate. Purification was done by flash column to get the 600 mg of corresponding amide with a percentage yield of 73%.

2-methoxy-N-(4-methoxyphenyl)-6-nitroacridin-9-amine (RK-2) (68):

N-(4-methoxyphenyl)-2-(4-methoxyphenylamino)-4-nitrobenzamide (**67**) (393 mg, 1.00 mmol) is dissolved in 15ml of DCM and to this stirring solution 2 ml of $(COCl)_2$ was added and the resulting reaction mixture was stirred about 2 h at room temperature. Organic layer was washed with sodium bicarbonate solution and did a column chromatography to get the 58.2 mg of pure 2-methoxy-N-(4-methoxyphenyl)-6-nitroacridin-9-amine (RK-2) (**68**) with a percentage yield 0f 15%.

2.7 Spectral Section:

2-(4-methoxyphenylamino)-4-nitrobenzoic acid (66): ^{1}H NMR (dmso): δ 9.57 (b, 1 H), 8.06 (d, *J* = 8.4 Hz, 1 H), 7.53 (s, 1 H), 7.4 (d, *J* = 8.8 Hz, 1 H), 7.25 (d, *J* = 7.2 Hz, 2 H), 7.01 (d, *J* = 6.8 Hz, 2 H), 3.77 (s, 3 H) ppm; ^{13}C NMR (dmso): δ 169.5, 157.7, 151.6, 150.2, 134.3, 132.2, 126.7, 116.6, 115.7, 110.4, 107.5, 56.0 ppm.

N-(4-methoxyphenyl)-2-(4-methoxyphenylamino)-4-nitrobenzamide (67): ^{1}H NMR ($CDCl_3$): δ 9.20 (b, 1 H), 7.90 (d, *J* = 2.0 Hz, 1 H), 7.64 (d, *J* = 8.8 Hz, 1 H), 7.49-7.44 (m, 3 H), 7.14 (d, *J* = 8.8 Hz, 2 H), 6.94-6.91 (m, 4 H), 3.82 (s, 6 H) ppm; ^{13}C NMR ($CDCl_3$): δ 166.7, 157.5, 157.4, 150.9, 149.0, 132.4, 130.2, 128.7, 125.6, 124.7, 123.1, 121.2, 115.5, 115.3, 114.6, 110.7, 108.7, 55.8 ppm.

9-Aminoacridine (52a): ^{1}H NMR (dmso): δ 8.48 (d, *J* = 8.8 Hz, 2 H), 7.92 (d, *J* = 8.4 Hz, 2 H), 7.64 (t, *J* = 7.2 Hz, 2 H), 7.31 (t, *J* = 7.2 Hz, 2 H), 3.97-3.80(b, 2 H) ppm; ^{13}C NMR (dmso): δ 151.0, 149.5, 130.7, 129.3, 124.1, 122.3, 113.7 ppm.

2-(Methylacridin)-9-amine (52b): ^{1}H NMR (dmso): δ 8.38 (d, *J* = 8.4 Hz, 1 H), 8.18 (s, 1 H), 7.81 (d, *J* = 8.4 Hz, 1 H), 7.75 (d, *J* = 8.4 Hz, 1 H), 7.67 (b, 2 H), 7.59 (t, *J* = 6.8 Hz, 1 H), 7.47 (d, *J* = 8.4 Hz, 1 H), 7.27 (t, *J* = 6.8 Hz, 1 H), 2.45 (s, 3 H) ppm; ^{13}C NMR (dmso): δ 149.9, 149.1, 148.3, 133.0, 131.4, 130.1, 129.4, 129.3, 123.9, 122.2, 122.2, 113.7, 113.5, 22.1 ppm.

4-(2-(2-methoxyethoxy)ethoxy)aniline (62): ^{1}H NMR ($CDCl_3$): δ 6.58 (d, *J* = 8.8 Hz, 2 H), 6.45 (d, *J* = 8.8 Hz, 2 H), 3.87 (t, *J* = 4.8 Hz, 2 H), 3.63 (t, *J* = 4.8 Hz, 2 H), 3.57 (b, 2 H), 3.53 (t, *J* = 4.8 Hz, 2 H), 3.40 (t, *J* = 4.8 Hz, 2 H), 3.22 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 151.8, 140.6, 116.56, 115.9, 72.0, 70.6, 70.0, 68.2, 59.0 ppm.

4-(acridin-9-ylamino)-4-oxobut-2-enoic acid (53a): ^{1}H NMR (dmso): δ 9.80-9.98 (b, 2 H), 8.56 (d, *J* = 8.4 Hz, 2 H), 7.97 (t, *J* = 7.6 Hz, 2 H), 7.79 (d, *J* = 8.8 Hz, 2 H), 7.54 (t, *J* = 7.6 Hz, 2 H), 6.05 (s, 2 H) ppm; ^{13}C NMR (dmso): δ 168.0, 158.5, 140.0, 136.9, 136.3, 125.3, 124.6, 119.5, 112.2 ppm.

4-(2-methylacridin-9-ylamino)-4-oxobut-2-enoic acid (53b): ^{1}H NMR (dmso): δ 9.79 (b, 2 H), 8.59 (d, *J* = 8.8 Hz, 1 H), 8.42 (s, 1 H), 7.99 (t, *J* = 7.2 Hz, 1 H), 7.84 (q, *J* = 15.2 Hz, 6.4, 2 H), 7.76 (d, *J* = 8.8 Hz, 1 H), 7.57 (t, *J* = 7.6 Hz, 1 H), 6.00 (s, 2 H), 2.51 (s, 3 H) ppm; ^{13}C NMR (dmso): δ 168.2, 157.4, 139.4, 137.9, 137.8, 136.9, 135.7, 134.0, 125.0, 124.2, 123.5, 119.3, 119.0, 112.0, 21.6 ppm.

2-(acridin-9-ylcarbamoyl)benzoic acid (60a): ^{1}H NMR (dmso): δ 9.80-9.93 (b, 2 H), 8.61 (d, *J* = 8.4 Hz, 2 H), 8.13 (q, *J* = 5.6, 2.0 Hz, 2 H), 8.00 (t, *J* = 8.0 Hz, 2 H), 7.84 (d, *J* = 8.4 Hz, 2 H), 7.58 (t, *J* = 8.0 Hz, 2 H), 7.48-7.46 (m, 2 H) ppm; ^{13}C NMR (dmso): δ 169.1, 158.2, 139.9, 136.0, 135.6, 133.1, 131.1, 125.2, 124.4, 119.5, 112.1 ppm.

2-(2-methylacridin-9-ylcarbamoyl)benzoic acid (60b): ^{1}H NMR (dmso): δ 9.67-9.80 (b, 1 H), 8.56 (d, *J* = 8.8 Hz, 1 H), 8.36 (s, 1 H), 8.16 (q, *J* = 6.0, 2.4 Hz, 2 H), 7.95 (t, *J* = 7.6 Hz, 1 H), 7.80 (d, *J* = 8.4 Hz, 2 H), 7.72 (d, *J* = 8.8 Hz, 1 H), 7.53 (t, *J* = 7.6 Hz, 1 H), 7.48 (q, *J* = 6.0 Hz, 2.4, 2 H), 2.47 (s, 3 H) ppm; ^{13}C NMR (dmso): δ 169.3, 157.3, 139.5, 138.0, 137.7, 135.6, 133.9, 133.2, 131.1, 125.0, 124.1, 123.5, 119.4, 119.2, 111.8, 21.5 ppm.

4-(acridin-9-ylamino)-4-oxobutanoic acid (60): ^{1}H NMR (dmso): δ 8.48 (d, *J* = 8.4 Hz, 2 H), 7.84-7.77 (m, 4 H), 7.42 (t, *J* = 8.4 Hz, 2 H), 2.31 (s, 4 H) ppm; ^{13}C NMR (dmso): δ 175.3, 55.6, 143.7, 134.1, 124.8, 123.7, 123.3, 112.7, 31.4 ppm.

N-(4-(2-(2-methoxyethoxy)ethoxy)phenyl)acridin-9-amine (RK-1) (63): ^{1}H NMR ($CDCl_3$): δ 8.47 (s, 1 H), 8.15 (d, *J* = 8.4 Hz, 1 H), 7.86 (s, 1 H), 7.81 (d, *J* = 8.4 Hz, 1 H), 7.37 (d, *J* = 8.8 Hz, 1 H), 7.24 (d, *J* = 8.8 Hz, 2 H), 6.90 (d, *J* = 8.8 Hz, 2 H), 6.81 (d, *J* = 8.8 Hz, 2 H), 3.79 (s, 3 H), 3.77 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 164.1, 163.2, 159.6, 157.16, 149.2, 141.3, 138.2, 133.3, 130.4, 129.8, 127.3, 127.1, 124.1, 123.1, 122.6, 122.4, 121.7, 121.5, 115.4, 114.8, 114.53, 114.5, 114.4, 114.3, 55.8, 55.7 ppm.

2-methoxy-N-(4-methoxyphenyl)-6-nitroacridin-9-amine (RK-2) (68): ^{1}H NMR ($CDCl_3$): δ 7.95 (d, *J* = 8.0 Hz, 2 H), 7.80 (b, 2 H), 7.53 (t, *J* = 6.8 Hz, 2 H), 7.14 (t, *J* = 6.8 Hz, 2 H), 6.85-6.79 (m, 4 H), 4.09 (t, *J* = 4.4 Hz, 2 H), 3.84 (t, *J* = 5.2 Hz, 2 H), 3.72 (t, *J* = 4.0 Hz, 2 H), 3.58 (t, *J* = 5.2 Hz, 2 H), 3.37 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 154.6, 130.7, 125.3, 123.1, 120.5, 119.3, 115.8, 77.6, 77.3, 77.0, 72.2, 71.0, 70.1, 68.0, 59.3 ppm.

1-(acridin-9-yl)-1*H*-pyrrole-2,5-dione (RK-3) (54a): ^{1}H NMR (dmso): δ 8.27 (d, *J* = 8.8 Hz, 2 H), 7.97 (d, *J* = 8.8 Hz, 2 H), 7.93 (t, *J* = 8.0 Hz, 2 H), 7.67 (t, *J* = 8.0 Hz, 2 H), 7.47 (s, 2 H) ppm; ^{13}C NMR (dmso): δ 170.8, 149.5, 136.2, 134.7, 131.7, 130.2, 128.4, 124.5, 124.1, 36.0 ppm.

1-(acridin-9-yl)-3-(phenylthio)pyrrolidine-2,5-dione (RK-4) (56a): ^{1}H NMR (dmso): δ 8.30 (d, *J* = 8.8 Hz, 2 H), 7.78 (t, *J* = 8.8 Hz, 2 H), 7.66 (d, *J* = 8.4 Hz, 2 H), 7.61 (d, *J* = 8.4 Hz, 1 H), 7.48 (p, *J* = 6.4 Hz, 3 H), 7.40 (t, *J* = 7.6 Hz, 2 H), 7.12 (d, *J* = 8.8 Hz, 1 H), 4.47 (q, *J* = 9.2 Hz, 4.0, 1 H), 3.60 (q, *J* = 9.2 Hz, 9.2, 1 H), 3.19 (d, *J* = 19.2 Hz, 1 H) ppm; ^{13}C NMR (dmso): δ 174.2, 173.5, 149.6, 148.6, 135.9, 133.8, 130.7, 130.6, 130.4, 130.2, 130.1, 130.0, 127.9, 127.9, 123.0, 122.9, 122.2, 122.2, 45.5, 36.7 ppm.

9-(phenylthio)acridine (RK-5) (57a): ^{1}H NMR (dmso): δ 8.69 (d, *J* = 8.8 Hz, 2 H), 8.29 (d, *J* = 8.8 Hz, 2 H), 7.78 (t, *J* = 7.6 Hz, 2 H), 7.54 (t, *J* = 7.6 Hz, 2 H), 7.11-7.06 (m, 3 H), 7.00 (d, *J* = 8.4 Hz, 2 H) ppm; ^{13}C NMR (dmso): δ 149.3, 139.4, 137.2, 130.6, 130.4, 129.4, 129.3, 129.2, 127.7, 127.5, 127.4, 127.0, 126.1 ppm.

1-(2-methylacridin-9-yl)-1*H*-pyrrole-2,5-dione (RK-6) (54b): ^{1}H NMR (dmso): δ 8.31 (d, *J* = 8.8 Hz, 1 H), 8.22 (d, *J* = 8.8 Hz, 1 H), 7.79 (t, *J* = 7.6 Hz, 1 H), 7.66 (d, *J* = 7.6 Hz, 2 H), 7.58 (t, *J* = 7.6 Hz, 1 H), 7.40 (s, 1 H), 7.14 (s, 2 H), 2.56 (s, 3 H) ppm; ^{13}C NMR (dmso): δ 169.5, 138.2, 135.2, 133.8, 130.5, 130.2, 127.7, 124.4, 124.4, 122.4, 120.5, 22.5 ppm.

N- (9-Acridinyl)succinimide(NAS) (RK-7) (59): ^{1}H NMR (dmso): δ 8.27 (d, *J* = 8.8 Hz, 2 H), 8.12 (d, *J* = 8.4 Hz, 2 H), 7.92 (t, *J* = 7.2 Hz, 2 H), 7.67 (t, *J* =7.6 Hz, 2 H), 3.17 (s, 4 H); ^{13}C NMR (dmso): δ 178.1, 149.5, 136.2, 131.7, 130.1, 128.1, 124.4, 123.6, 30.3 ppm.

1-(acridin-9-yl)-3-(ethylthio)pyrrolidine-2,5-dione (RK-8) ^{1}H NMR (dmso): δ 8.28 (t, *J* = 7.6 Hz, 2 H), 8.20 (d, *J* = 8.8 Hz, 1 H), 7.98-7.91 (m, 3 H), 7.75-7.67 (m, 2 H), 4.50 (d, *J* = 5.2 Hz, 1 H), 3.78 (q, *J* = 18.4 Hz, 8.8, 1 H), 3.07 (d, *J* = 18.4 Hz, 1 H), 2.89 (q, *J* =14.4 Hz, 7.2, 2 H), 1.29 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR (dmso): δ 176.9, 175.3, 149.5, 149.4, 135.1, 131.8, 131.7, 130.4, 130.1, 128.6, 128.2, 124.4, 123.7, 123.3, 122.8, 37.5, 26.1, 14.9 ppm.

3-(ethylthio)-1-(2-methylacridin-9-yl)pyrrolidine-2,5-dione (RK-9) ^{1}H NMR (dmso): δ 8.25 (t, *J* = 7.6 Hz, 1 H), 8.17 (t, *J* = 8.4 Hz, 1 H), 7.79 (q, *J* = 14.8, 8.2 Hz, 2 H), 7.77-7.66 (m, 3 H), 4.50 (t, *J* = 8.0 Hz, 1 H), 3.83-3.75 (m, 1 H), 3.05 (d, *J* = 18.4 Hz, 1 H), 2.90 (q, *J* = 19.2 Hz, 11.6, 2 H), 2.48 (s, 3 H), 1.28 (t, *J* = 11.2 Hz, 3 H); ^{13}C NMR (dmso): δ 176.9, 176.8, 175.4, 175.4, 148.9, 148.8, 148.6, 148.5, 138.4, 138.3, 134.6, 134.4, 133.0, 131.2, 131.1, 130.4, 130.2,

130.1, 129.9, 128.4, 128.0, 124.3, 123.8, 123.7, 123.4, 123.4, 122.7, 121.9, 120.7, 75.8, 37.5, 26.0, 25.9, 22.4, 22.3, 14.9, 14.9 ppm.

2-(acridin-9-yl)isoindoline-1,3-dione (RK-10) (61a): ^{1}H NMR (dmso): δ 8.29 (d, *J* = 8.8 Hz, 2 H), 8.12- 8.07 (m, 4 H), 8.02 (d, *J* = 8.4 Hz, 2 H), 7.93 (t, *J* = 7.6 Hz, 2 H), 7.64 (t, *J* = 7.6 Hz, 2 H); ^{13}C NMR (dmso): δ 167.9, 149.5, 135.9, 135.1, 132.5, 131.7, 130.2, 128.4, 124.8, 124.4, 124.3 ppm

2-(2-methylacridin-9-yl)isoindoline-1,3-dione (RK-11) (61b): ^{1}H NMR ($CDCl_3$): δ 8.26 (d, *J* = 8.8 Hz, 1 H), 8.19 (d, *J* = 9.2 Hz, 1 H), 8.11 (q, *J* = 5.2 Hz, 2.8, 2 H), 8.04-7.99 (m, 3 H), 7.88 (t, *J* = 9.2 Hz, 2 H), 7.77 (d, *J* = 8.8 Hz, 1 H), 7.62 (t, *J* = 7.6 Hz, 1 H), 2.48 (s, 3 H); ^{13}C NMR ($CDCl_3$): 168.0, 148.9, 148.6, 138.5, 135.8, 134.6, 133.9, 132.6, 131.1, 130.2, 129.9, 128.3, 124.8, 124.5, 124.4, 124.1, 122.0, 22.1.

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Chapter-3: Copper-Nicotinic Acid Complex Mediated Coupling of Aryl Halides With Nitrogen Nucleophiles

3.1 Introduction:

Transition metal catalyzed C-N cross coupling reactions are considered to be extremely powerful and versatile methods in the synthesis of pharmaceuticals, optical devices, and materials.[1] The conventional route for N- arylation of N-nucleophiles with aryl halides is the Ullmann type coupling reactions.[2] However they associated with the limitations of harsh conditions, and long reaction times. Great efforts have been made to minimize these problems, and one of them is Buchwald – Hartwig amination.[3] The reaction requires palladium-based catalysts with phosphine or N-heterocyclic carbene ligands and proceeds under milder conditions. We also have various reports of Pd catalyzed C-N bond formation by coupling aryl halides with amines,[4] amides [5] and imides.[6] However the high cost of Pd and use of external ligands makes it difficult to apply these reactions in the industrial scale.

Recently there has been a significant development in the copper catalysis, especially in the formation of C-N bond by using external ligands such as diamines,[7] diols,[8] triols,[9] rac-binols,[10] salicylamides,[11] β-diketones,[12] β-ketoesters,[13] imines,[14] amino acids,[15] amino phosphates,[16] and diazaphospholanes.[17] However, most of these ligands are hard to synthesize and these catalytic systems are not applicable to all kind of N-nucleophiles. Recently we reported a ligand free copper (II) catalyzed formamidation of aryl halides. So we are in a desperate need of common catalytic system that can be applicable to all kind of N- Nucleophiles. Here we are

reporting a copper- nicotinic acid complex catalytic system that applicable to coupling of aryl halides with various N-nucleophiles such as N-formyl amines and cyclic imides.

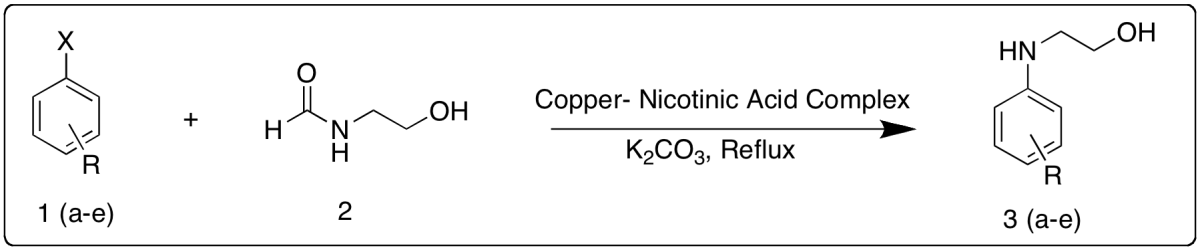
3.2 Results and Discussion:

We synthesized the copper-nicotinic acid complex by stirring equivalent amounts of $CuSO_4 \bullet 5H_2O$ and nicotinic acid in DMF at 150°C about over night. The formed dirty red color precipitate was filtered off from the reaction mixture and washed with water and dried. However, we were unable to derive its actual structure so we assumed that Cu and nicotinic acid were boned together in 1:1 ratio.

We optimized the reaction conditions by stirring the mixture of bromobenzene, N-formyl ethanolamine, potassium carbonate and copper-nicotinic acid complex under refluxing conditions for 4h (Scheme 3.1).

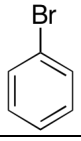
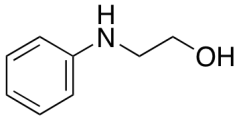
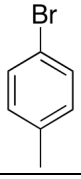
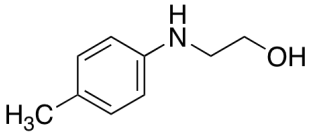
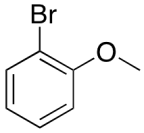
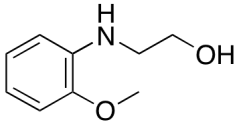
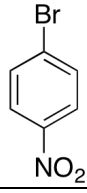
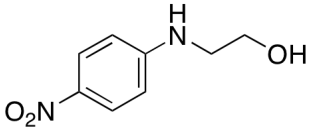
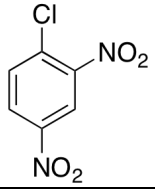
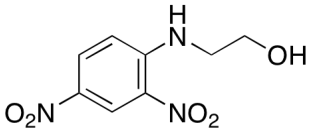
At first we tested the catalyst effect by loading 10%, and ended up seeing only 10% conversion of the product. So we concluded that the reaction requires equivalent amounts copper complex. With an established condition for coupling reaction, we then examined the scope of the process to other N-formy amines. We noticed that the reaction works very well with. When N-formyl butylamine was chosen, significant increase in the reaction timings were observed, and this is because of low boiling point of N-formyl butylamine. It is important to mention that the reported aliphatic N-formyl amines are all liquids, therefore the reactions doesn't require any additional solvent systems.

3.2.1 Aryl halides coupling with N-formyl-2- aminoethanol

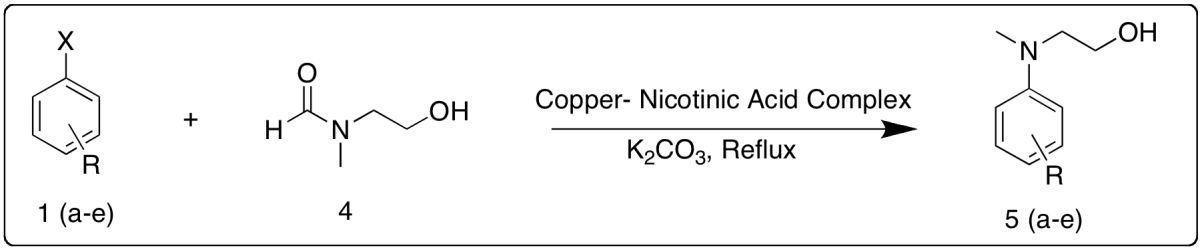


Scheme 3.1 Aryl halides coupling with N-formyl-2-aminoethanol

Table 3.1 Prepared of N-aryl-2-ethanolamines

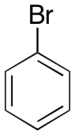
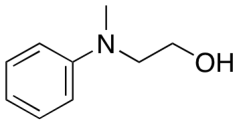
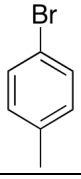
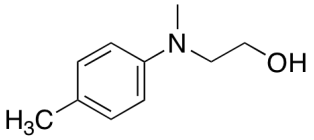
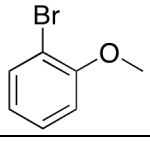
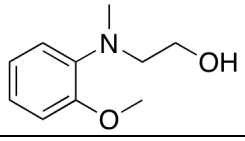
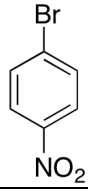
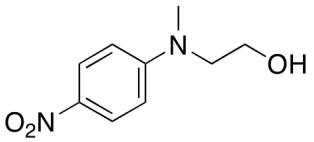
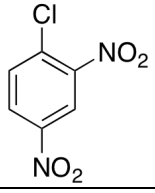
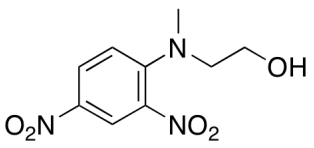
Entry No	Aryl Halide	Product Structure	Reaction Time (h)	Yield (%)
A			4	82
b			4	80
c			4	91
d			4	94
e			4	85

3.2.2 Aryl halides coupling with N-formyl-2-(methylamino)ethanol

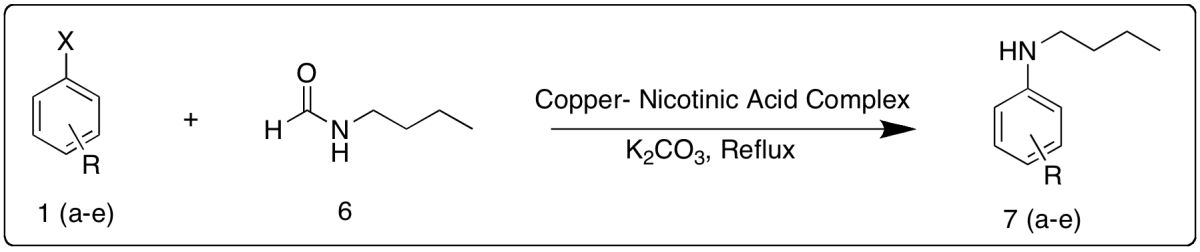


Scheme 3.2 Aryl halides coupling with N-formyl-2-(methylamino)ethanol

Table 3.2 Prepared Methyl- N-aryl ethanolamines

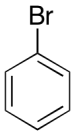
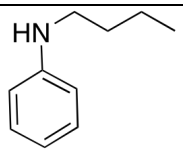
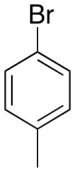
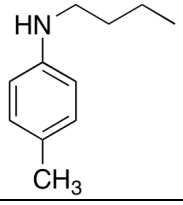
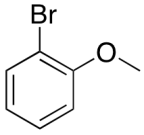
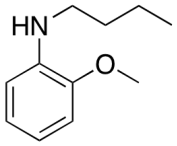
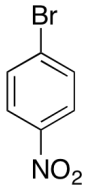
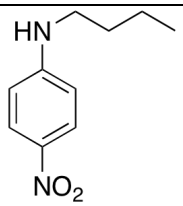
Entry No	Aryl Halide	Product Structure	Reaction Time (h)	Yield (%)
a			4	81
b			5	86
c			5	79
d			3	91
e			2	84

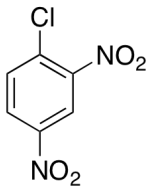
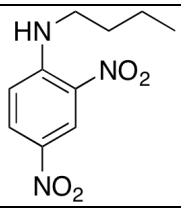
3.2.3 Aryl halides coupling with N-formyl butylamine:



Scheme 3.3 Aryl halides coupling with N-formyl butylamine

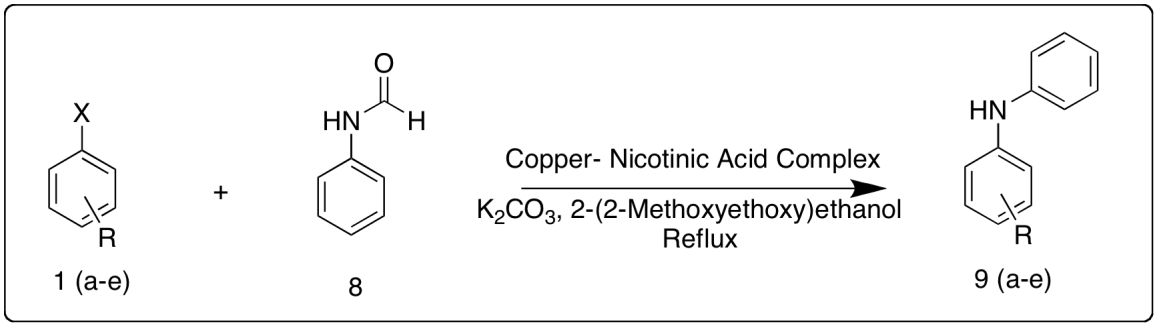
Table 3.3 Preparation of N-Arylbutylamines

Entry No	Aryl Halide	Product Structure	Reaction Time (h)	Yield (%)
a			24	72
b			24	77
c			24	83
d			18	86

e			12	93

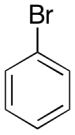
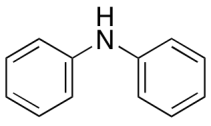
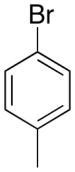
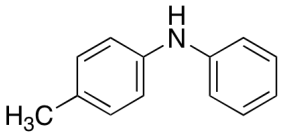
Where as N-formyl amine requires an additional solvent such as DMF or 2(2-Methoxy-ethoxy ethanol). However 2(2-Methoxy-ethoxy ethanol) is the ideal solvent as it takes relatively less time to complete the reaction. We also explored the scope of the reaction with respect to aryl halides, and resulted with excellent yields irrespective of the nature of the aryl halides.

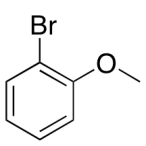
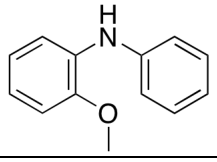
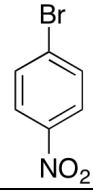
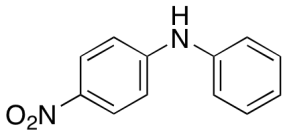
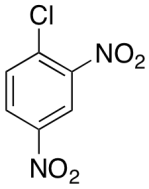
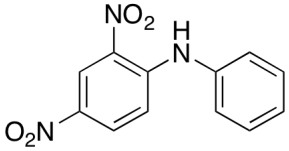
3.2.4 Aryl halides coupling with N-Formyl aniline:



Scheme 3.4 Aryl halides coupling with N-formyl aniline

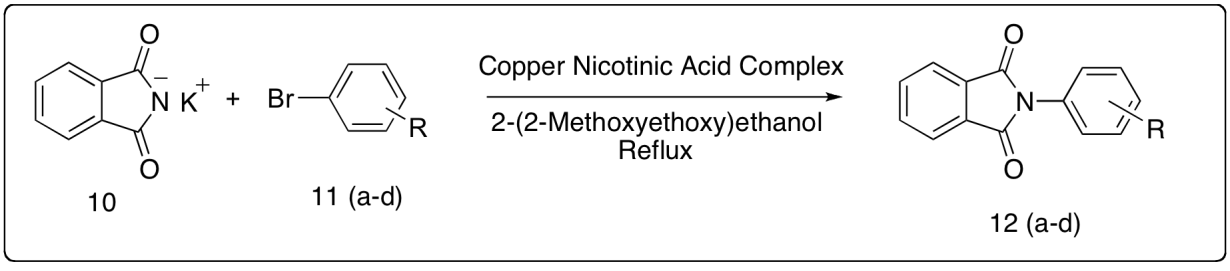
Table 3.4 Preparation of N-arylanilines

Entry No	Aryl Halide	Product Structure	Reaction Time (h)	Yield (%)
a			18	91
b			24	80

c			12	92
d			12	81
e			12	90

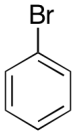
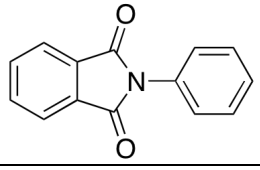
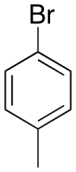
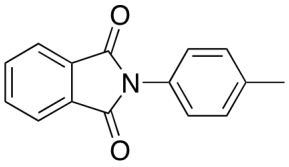
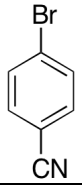
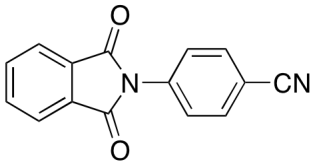
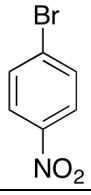
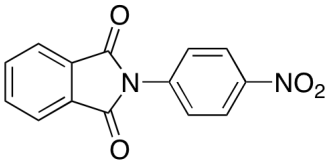
Furthermore, we investigated the coupling reaction of aryl bromides with various cyclic imides such as phthalimide, naphthylimide, succinimide, and dimethyl hydantoin. First we tested the reaction conditions with bromobenzene, phthalimide, potassium carbonate, and Copper complex in DMF as the reaction takes 4h to complete. We observed significant decrease in reaction timings (3h) when we started with Potassium salt of phthalimide as substrate and 2(2methoxy ethoxy ethanol) as solvent.

3.2.5 Potassium phthalimide coupling with aryl halides

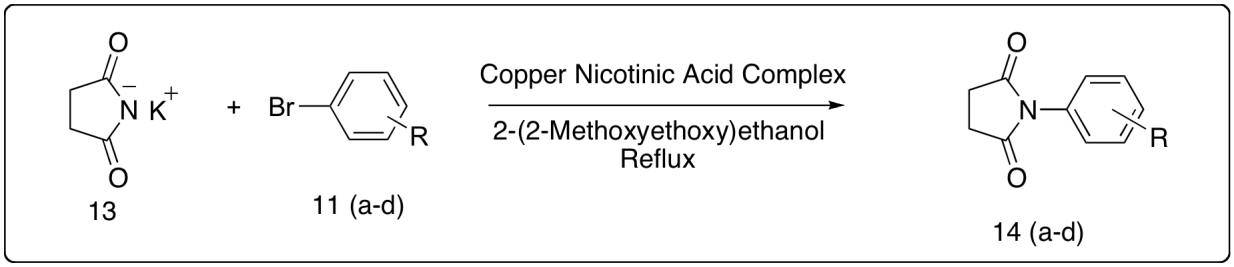


Scheme 3.5 Potassium Phthalimide coupling with Aryl halides

Table 3.5 Preparation of N-Aryl Phthalimides

Entry No	Aryl Halide	Product Structure	Reaction Time (h)	Yield (%)
a			3	93
b			3	91
c			4	95
d			4	89

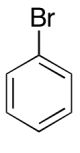
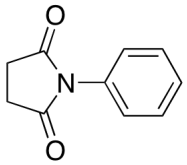
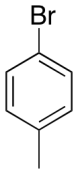
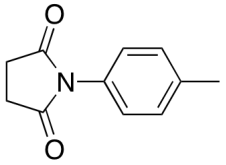
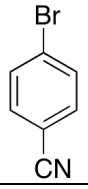
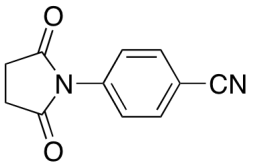
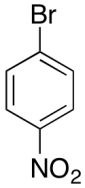
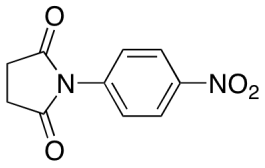
3.2.6 Potassium succinimide coupling with aryl halides



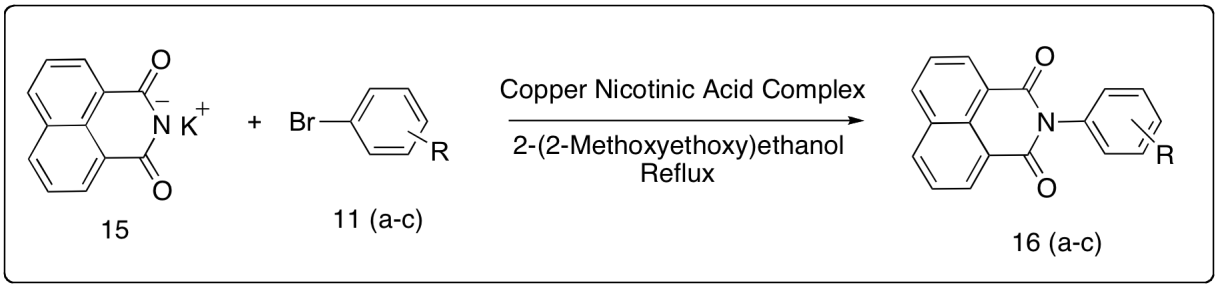
Scheme 3.6 Potassium Succinimide coupling with Aryl halides

Table 3.6 Preparation of N-Aryl succinimides

Entry No	Aryl Halide	Product Structure	Reaction Time (h)	Yield (%)

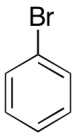
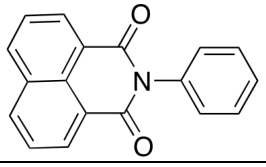
a			4	89
b			4	91
c			3	93
d			3	91

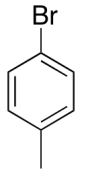
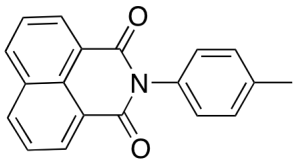
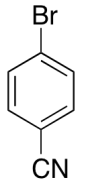
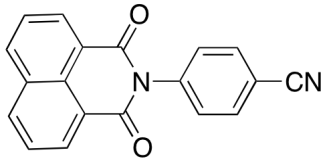
3.2.7 Potassium 1,8-naphthylimide coupling with aryl halides



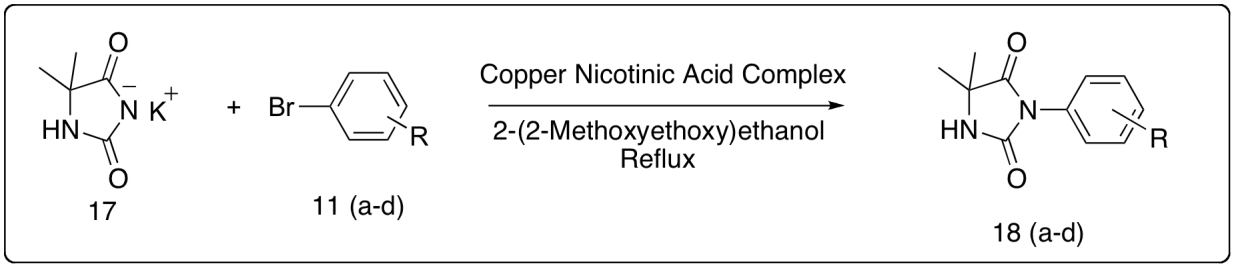
Scheme 3.7 Potassium Naphthylimide coupling with Aryl halides

Table 3.7 Preparation of N-Aryl naphthylimides

Entry No	Aryl Halide	Product Structure	Reaction Time (h)	Yield (%)
a			4	91

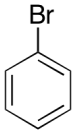
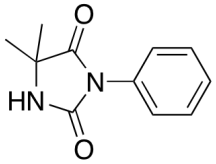
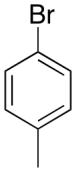
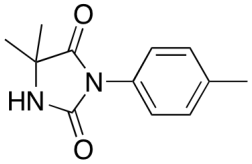
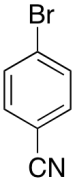
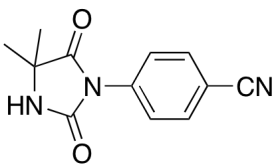
b			4	94
c			4	86

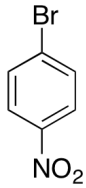
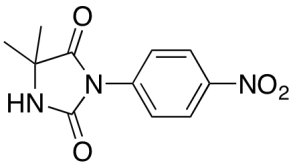
3.2.8 Potassium 5,5-dimethyl hydantoin coupling with aryl halides:



Scheme 3.8 Potassium 5,5-dimethylhydantoin coupling with aryl halides

Table 3.8 Preparation of N-aryl dimethylhydantoins

Entry No	Aryl Halide	Product Structure	Reaction Time (h)	Yield (%)
a			2.5	94
b			2	93
c			2	89

d			2	91

3.3 Conclusions:

In conclusion we developed a copper nicotinic acid complex that catalyze the coupling of aryl halides with various nitrogen nucleophiles such as N-formyl amines and cyclic imides. Most of the reactions not even required of any additional solvent. The other advantages like short reaction times, moderate to excellent yields and variety of substrate tolerability makes this method versatile to form the C-N bond.

3.4 Experimental Section:

Synthesis of Copper-Nicotinic acid complex: $CuSO_4 \bullet 5H_2O$ (5.0 g, 0.020 mol) was dissolved in 100 ml of DMF and to this solution (2.46 g, 0.020 mol) of 2-nicotinic acid and (2.78 g, 0.02 mol) of potassium carbonate were added. The whole reaction mixture was stirred under refluxing conditions about over night. The formed dirty red precipitate filtered off from the reaction mixture and washed with water and dried. The dirty red powder was used without further purification.

General procedure for N-formylation of amines: Synthesis of N-formyl-2-ethanolamine (2):

(30.5 g, 0.500 mol) of ethanolamine was dissolved in (53.6 g, 0.500 mol) of trimethyl orthoformate and to this solution (2.0 g, 0.012 mol) *p*-toluenesulfonic acid and few drops of water is added, the whole reaction mixture is stirred in a microwave at a power of 200 about over night. Bring down the temperature to room temperature and the formed N-formy-2-ethanolamine was used directly for the coupling reactions without further purification.

General procedure for coupling of aryl halides with N-formyl-2-ethanolamine:

Synthesis of 2-(*p*-tolylamino)ethanol (3b):

p-bromotoluene (342 mg, 2.00 mmol) , potassium carbonate (306 mg, 2.20 mmol) and (372 mg, 2.00 mmol) of copper-nicotinic acid complex were dissolved in 12 ml of N-formyl-2-ethanolamine and the whole reaction mixture was stirred at 150°C about 4 h and monitored the reaction progress by TLC. After completion of the reaction cool it down to the room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 248 mg of 2-(*p*-tolylamino)ethanol (**3c**) with 82% yield.

General procedure for aryl halides coupling with N-formyl-2-(methylamino)ethanol:

Synthesis of 2-(methyl(*p*-tolyl)amino)ethanol (5b):

p-bromotoluene (342 mg, 2.00 mmol) , (306 mg, 2.20 mmol) of potassium carbonate and (372 mg, 2.00 mmol) of copper-nicotinic acid complex were dissolved in 12 ml of N-formyl-2-(methylamino)ethanol and the whole reaction mixture was stirred at 160°C about 5 h and monitored the reaction progress by TLC. After completion of the reaction cool it down to the

room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 284 mg of 2-(methyl(*p*-tolyl)amino)ethanol (**5b**) with 86% yield.

General procedure for aryl halides coupling with N-formylbutylamine:

Synthesis of N-butyl-4-methylaniline (7b):

p-bromotoluene (342 mg, 2.00 mmol) , (306 mg, 2.20 mmol) of potassium carbonate and (372 mg, 2.00 mmol) of copper-nicotinic acid complex were dissolved in 12 ml of N-formylbutylamine and the whole reaction mixture was stirred at 100°C about 24 h and monitored the reaction progress by TLC. After completion of the reaction cool it down to the room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 251 mg of N-butyl-4-methylaniline (**7b**) with 77% yield.

General procedure for aryl halides coupling with N-formylaniline:

Synthesis of 4-methyl-N-phenylaniline (9b):

N-formyl aniline (66.0 mg, 2.20 mmol) was dissolved in 10 ml of 2-(2-methoxyethoxy)ethanol, to this stirring solution (342 mg, 2.00 mmol) of *p*-bromotoluene, (306 mg, 2.20 mmol) of potassium carbonate and (372 mg, 2.00 mmol) of copper-nicotinic acid complex were added and the whole reaction mixture was stirred at refluxing conditions about 24 h and monitored the reaction progress by TLC, after completion of reaction cool it down to the room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted

the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 293 mg of 4-methyl-N-phenylaniline (**9b**) with 80% yield.

General preparation of potassium salts of cyclic imides: Preparation of potassium phthalimide (10):

Hot ethanol (30 ml) solution of phthalimide (1470 mg, 10.00 mmol) and aqueous ethanol (1 ml water plus 3 ml ethanol) solution potassium hydroxide (560 g, 10.0 mmol) were mixed together. Resulting solution was left at room temperature for one hour. Formed white precipitate was separated by filtration, washed with ice-cold ethanol (3x5 ml) and dried on air to give pure product in approximately 93% isolated yield.

General procedure for aryl halides coupling with potassium phthalimide: Synthesis of 2-phenylisoindoline-1, 3-dione (12a): To a 2-(2-methoxyethoxy)ethanol suspension of Potassium Phthalimide (222 mg, 1.20 mmol) was added bromobenzene (157 mg, 1.00 mmol) and copper-nicotinic acid complex (186 mg, 1.00 mmol). The reaction mixture was under refluxing conditions for 4 h and monitored the completion reaction by TLC. After the reaction is complete the reaction mixture was cooled to room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 207 mg of 2-phenylisoindoline-1, 3-dione (**12a**) with 93% yield.

General procedure for aryl halides coupling with potassium succinimide: Preparation of 1-phenylpyrrolidine-2,5-dione (14a): To a 2-(2-methoxyethoxy)ethanol suspension of Potassium Succinimide (164 mg, 1.20 mmol) was added Bromo Benzene (157 mg, 1.00 mmol) and copper-nicotinic acid complex (186 mg, 1.00 mmol). The reaction mixture was stirred under refluxing conditions for 4 h and monitored the completion reaction by TLC. After the reaction is complete

the reaction mixture was cooled to room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 156 mg of 1-phenylpyrrolidine-2,5-dione with 89% yield.

General procedure for aryl halides coupling with potassium-1,8-naphthalimide: Synthesis of 2-phenyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (16a): To a 2-(2-methoxyethoxy)ethanol suspension of Potassium salt of 1,8 Naphthalimide (282 mg, 1.20 mmol) was added Bromo Benzene (157 mg, 1.00 mmol) and copper-nicotinic acid complex (186 mg, 1.00 mmol). The reaction mixture was stirred under refluxing conditions for 4 h and monitored the completion reaction by TLC. After the reaction is complete the reaction mixture was cooled to room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 248 mg of 2-phenyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione with 91% yield.

General procedure for aryl halides coupling with potassium-5,5-dimethylhydantoin: Synthesis of 5,5-dimethyl-3-phenylimidazolidine-2,4-dione(18a): To 2-(2-methoxyethoxy)ethanol a suspension of Potassium salt of 5,5- dimethyl Hydantoin (166 mg, 1.0 0 mmol) was added Bromo Benzene (157 mg, 1.0 mmol) and copper-nicotinic acid complex (186 mg, 1.0 mmol). The reaction mixture was stirred under refluxing conditions for 2.5 h and monitored the completion reaction by TLC. After the reaction is complete the reaction mixture was cooled to room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried

under sodium sulfate and a flash column required getting 192 mg of 5,5-dimethyl-3-phenylimidazolidine-2,4-dione with a 94% yield.

3.5 Spectral Section:

2-(phenylamino)ethanol (3a): ^{1}H NMR ($CDCl_3$): δ 7.20 (t, *J* = 7.2 Hz, 2 H), 6.76 (t, *J* = 7.2 Hz, 1 H), 6.69 (d, *J* = 8.0 Hz, 2 H), 3.84 (t, *J* = 4.4 Hz, 2 H), 3.32 (t, *J* = 5.2 Hz, 2 H), 2.72 (b, 2 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.1, 129.6, 118.5, 113.7, 61.4, 46.6 ppm.

2-(*p*-tolylamino)ethanol (3b): ^{1}H NMR ($CDCl_3$): δ 7.01 (d, *J* = 8.0 Hz, 2 H), 6.59 (d, *J* = 8.0 Hz, 2 H), 3.79 (t, *J* = 4.8 Hz, 2 H), 3.25 (t, *J* = 5.2 Hz, 2 H), 3.08 (b, 2 H), 2.26 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 146.1, 130.1, 127.5, 113.8, 61.4, 46.8, 20.6 ppm.

2-(2-methoxyphenylamino)ethanol (3c): ^{1}H NMR ($CDCl_3$): δ 6.88 (t, *J* = 7.6 Hz, 1 H), 6.79 (d, *J* = 7.6 Hz, 1 H), 6.72 (d, *J* = 8.0 Hz, 1 H), 6.68 (t, *J* = 6.0 Hz, 1 H), 3.84 (t, *J* = 5.6 Hz, 2 H), 3.32 (t, *J* = 4.8 Hz, 2 H) ppm; ^{13}C NMR ($CDCl_3$): δ 147.4, 138.1, 121.5, 117.4, 110.6, 109.8, 61.5, 55.7, 46.2 ppm.

2-(4-nitrophenylamino)ethanol (3d): ^{1}H NMR ($CDCl_3$): δ 8.09 (d, *J* = 9.2 Hz, 2 H), 6.57 (d, *J* = 9.2 Hz, 2 H), 3.91 (t, *J* = 5.2 Hz, 2 H), 3.40 (t, *J* =4.8 Hz, 2 H), 1.63 (b, 2 H) ppm; ^{13}C NMR ($CDCl_3$): δ 126.7, 111.6, 61.1, 45.4 ppm.

2-(2,4-dinitrophenylamino)ethanol (3e): ^{1}H NMR ($CDCl_3$) : δ 9.14 (s, 1 H), 8.82 (b, 1 H), 8.28 (d, *J* = 9.6 Hz, 1 H), 6.98 (d, *J* = 9.6 Hz, 1 H), 4.02 (t, *J* = 5.6 Hz, 2 H), 3.6 (q, *J* = 10.8, 5.6 Hz, 2 H), 1.64 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.8, 130.6, 124.6, 114.3, 60.7, 45.4 ppm.

2-(methyl(phenyl)amino)ethanol (5a): ^{1}H NMR ($CDCl_3$): 7.26 (t, *J* = 7.6 Hz, 2 H), 6.82 (d, *J* = 8.4 Hz, 2 H), 6.77 (t, *J* = 7.2 Hz, 1 H), 3.81 (t, *J* = 5.6 Hz, 2 H), 3.47 (t, *J* = 5.6 Hz, 2 H), 2.97 (s, 3 H), 2.06 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 150.3, 129.5, 117.5, 113.4, 60.3, 55.7, 39.1 ppm.

2-(methyl(*p*-tolyl)amino)ethanol (5b): ^{1}H NMR ($CDCl_3$); δ 7.07 (d, *J* = 8.0 Hz, 2 H), 6.76 (d, *J* = 8.4 Hz, 2 H), 3.79 (t, *J* = 5.6 Hz, 2 H), 3.41 (t, *J* = 5.6 Hz, 2 H), 2.92 (s, 3 H), 2.28 (s, 3 H), 2.11 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.5, 130.0, 127.2, 114.1, 60.2, 56.3, 39.2, 20.5 ppm.

2-((2-methoxyphenyl)(methyl)amino)ethanol (5c): ^{1}H NMR ($CDCl_3$): δ 7.04 (t, *J* = 7.6 Hz, 1 H), 7.00 (d, *J* = 8.0 Hz, 1 H), 6.91 (t, *J* = 7.6 Hz, 1 H), 6.86 (d, *J* = 7.6 Hz, 1 H), 3.84 (s, 3 H), 3.71 (t, *J* = 5.2 Hz, 2 H), 3.24 (b, 1 H), 3.11 (t, *J* = 5.2 Hz, 2 H), 2.78 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 152.9, 142.1, 123.6, 121.2, 12.4, 111.5, 59.7, 58.4, 55.5, 40.1 ppm.

2-(methyl(4-nitrophenyl)amino)ethanol (5d): ^{1}H NMR ($CDCl_3$): δ 8.00 (d, *J* = 9.6 Hz, 2 H), 6.62 (d, *J* = 9.2 Hz, 2 H), 3.86 (t, *J* = 5.6 Hz, 2 H), 3.62 (t, *J* = 6.0 Hz, 2 H), 3.12 (s, 3 H), 2.2 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 154.1, 137.0, 126.4, 110.7, 60.2, 54.7, 39.7 ppm.

2-((2,4-dinitrophenyl)(methyl)amino)ethanol (5e): ^{1}H NMR ($CDCl_3$): δ 8.65 (s, 1 H), 8.19 (d, *J* = 9.2 Hz, 1 H), 7.22 (d, *J* = 9.6 Hz, 1 H), 3.91 (t, *J* = 5.2 Hz, 2 H), 3.61 (t , *J* = 5.2 Hz, 2 H), 2.99 (s, 3 H), 1.87 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 149.8, 136.8, 127.8, 124.2, 118.9, 59.5, 56.2, 40.8 ppm.

N-butylaniline (7a): ^{1}H NMR ($CDCl_3$): δ 7.19 (t, *J* = 8.4 Hz, 2 H), 6.71 (t, *J* = 7.2 Hz, 1 H), 6.62 (d, *J* = 8.4 Hz, 2 H), 3.6 (b, 1 H), 3.13 (t, *J* = 7.2 Hz, 2 H), 1.63 (q, *J* = 7.2 Hz, 2 H), 1.46 (Sextet, *J*= 15.2, 7.6 Hz, 2 H), 0.98 (t, *J* = 7.6 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.8, 129.5, 117.3, 112.9, 43.9, 31.9, 20.6, 14.2 ppm.

N-butyl-4-methylaniline (7b): ^{1}H NMR ($CDCl_3$): δ 7.08 (d, *J* = 7.6 Hz, 2 H), 6.63 (d, *J* = 8.4 Hz, 2 H), 3.46 (b, 1 H), 3.17 (t, *J* = 6.8 Hz, 2 H), 2.34 (s, 3 H), 1.68 (p, *J* = 6.8 Hz, 2 H), 1.53 (p, *J* = 7.6 Hz, 2 H), 1.06 (t, *J* = 7.2 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 146.7, 130.0, 126.6, 113.2, 44.4, 32.0, 20.6, 14.3 ppm.

N-butyl-2-methoxyaniline (7c): ^{1}H NMR ($CDCl_3$): δ 6.89 (t, *J* = 7.6 Hz, 1 H), 6.78 (d, *J* = 8.0 Hz, 1 H), 6.68 (d, *J* = 7.6 Hz, 1 H), 6.64 (t, *J* = 7.6 Hz, 1 H), 4.20 (b), 3.86 (s, 3 H), 3.14 (t, *J* = 7.2 Hz, 2 H), 1.66 (p, *J* = 7.6 Hz, 2 H), 1.47 (Sextet, *J* = 14.8 Hz, 7.6, 2 H), 0.98 (t, *J* = 7.2 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 147.0, 138.8, 121.6, 116.3, 110.0, 109.6, 55.6, 43.6, 31.9, 20.6, 14.2 ppm.

N-butyl-4-nitroaniline (7d): ^{1}H NMR ($CDCl_3$); δ 8.03 (d, *J* = 9.2 Hz, 2 H), 6.49 (d, *J* = 9.2 Hz, 2 H), 4.78 (b, 1 H), 3.17 (q, *J* = 12.8 Hz, 7.2, 2 H), 1.61 (p, *J* = 7.6 Hz, 2 H), 1.27 (Sextet, *J* = 15.2, 7.2 Hz, 2 H), 0.93 (t, *J* = 7.6 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 154.0, 137.6, 126.7, 111.1, 43.3, 31.3, 20.4, 14.0 ppm.

N-butyl-2,4-dinitroaniline (7e): ^{1}H NMR ($CDCl_3$) ; δ 9.03 (s, 1 H), 8.53 (b), 8.21 (d, *J* = 9.6 Hz, 1 H), 6.92 (d, *J* = 9.6 Hz, 1 H), 3.41 (q, *J* = 12.4 Hz, 7.2, 2 H), 1.75 (p, *J* = 7.2 Hz, 2 H), 1.48 (Sextet, *J* = 15.2 Hz, 8.0, 2 H), 0.98 (t, *J* = 7.2 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.7, 136.0, 130.5, 130.3, 124.4, 114.3, 43.6, 30.9, 20.3, 13.9 ppm.

Diphenylamine (9a): ^{1}H NMR ($CDCl_3$): δ 7.33 (t, *J* = 8.4 Hz, 4 H), 7.13 (d, *J* = 8.4 Hz, 4 H), 7.00 (d, *J* = 8.0 Hz, 2 H), 5.70 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 143.4, 129.7, 121.3, 118.1 ppm.

4-methyl-N-phenylaniline (9b): ^{1}H NMR ($CDCl_3$): δ 7.35 (t, *J* = 8.8 Hz, 2 H), 7.20 (d, *J* = 8.02 Hz, 2 H), 7.13-7.10 (m, 4 H), 7.01 (t, *J* = 7.2 Hz, 1 H), 5.60 (b, 1 H), 2.43 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 144.3, 140.7, 131.2, 130.2, 129.7, 120.7, 119.3, 117.2, 21.1 ppm.

2-methoxy-N-phenylaniline (9c): ^{1}H NMR ($CDCl_3$): δ 7.43 (t, *J* = 6.8 Hz, 1 H), 7.39 (d, *J* = 7.2 Hz, 2 H), 7.27 (d, *J* = 8.0 Hz, 2 H), 7.06 (t, *J* = 8.0 Hz, 1 H), 7.02-6.99 (m, 3 H), 6.35 (b, 1 H), 3.96 (s, 3 H), ppm; ^{13}C NMR ($CDCl_3$): δ 148.5, 143.0, 133.2, 129.6, 121.4, 121.1, 120.2, 118.8, 114.9, 110.8, 55.8 ppm.

4-nitro-N-phenylaniline (9d): ^{1}H NMR ($CDCl_3$): 8.12 (d, *J* = 9.2 Hz, 2 H), 7.39 (t, *J* = 7.6 Hz, 2 H), 7.21 (d, *J* = 7.6 Hz, 2 H), 7.17 (t, *J* = 6.4 Hz, 1 H), 6.94 (d, *J* = 9.2 Hz, 2 H), 6.34 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 150.4, 140.0, 139.7, 130.0, 126.5, 124.9, 122.2, 113.9 ppm.

2,4-dinitro-N-phenylaniline(9e): ^{1}H NMR ($CDCl_3$): δ 9.98(b, 1 H), 9.16 (s, 1 H), 8.16 (d, *J* = 6.0 Hz, 1 H), 7.51 (t, *J* = 7.6 Hz, 2 H), 7.39 (t, *J* = 7.2 Hz, 1 H), 7.31 (d, *J* = 7.2 Hz, 2 H), 7.17 (d, *J* = 9.6 Hz, 1 H), 1.60 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 147.4, 137.6, 136.9, 131.3, 130.5, 130.2, 130.0, 125.8, 124.3, 116.3 ppm.

2-phenylisoindoline-1, 3-dione (12a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.79 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.50 (t, *J* = 7.3 Hz, 2 H), 7.44 (dt, *J* = 7.6, 1.4 Hz, 1 H), 7.40 (dt, *J* = 2.7, 1.6 Hz, 1 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.5, 134.6, 132.0, 131.9, 129.3, 128.3, 126.8, 124.0 ppm.

2-*p*-tolylisoindoline-1,3-dione (12b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 5.4, 3.1 Hz, 2 H), 7.77 (dd, *J* = 5.4, 3.1 Hz, 2 H), 7.31 (s, 4 H), 2.41 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.7, 138.4, 134.6, 132.0, 130.2, 130.0, 129.9, 129.2, 126.9, 126.7, 126.6, 123.9, 21.5 ppm.

4-(1,3-dioxoisoindolin-2-yl)benzonitrile (12c): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (dd, *J* = 5.5, 3.1 Hz, 2 H), 7.84 (dd, *J* = 5.5, 3.1 Hz, 2 H), 7.80 (d, *J* = 8.7 Hz, 2 H), 7.69 (d, *J* = 8.7 Hz, 2 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.7, 136.2, 135.2, 133.2, 131.6, 126.7, 124.3, 118.5, 111.5 ppm.

2-(4-nitrophenyl)isoindoline-1,3-dione (12d): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 9.2 Hz, 2 H), 8.00 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.85 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.77 (d, *J* = 9.2 Hz, 2 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.6, 146.6, 137.8, 135.2, 131.6, 126.6, 124.7, 124.4 ppm.

1-phenylpyrrolidine-2,5-dione (14a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (t, *J* = 7.5 Hz, 2 H), 7.41 (d, *J* = 7.5 Hz, 1 H), 7.28 (d, *J* = 7.8 Hz, 2 H), 2.90 (s, 4 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ176.6, 132.2, 129.4, 128.9, 126.7, 28.6 ppm.

1-*p*-tolylpyrrolidine-2,5-dione (14b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.1 Hz, 1 H), 7.14 (d, *J* = 7.8 Hz, 1 H), 2.84 (s, 4 H), 2.37 (s, 3 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.7, 139.0, 130.1, 129.5, 129.4, 128.9, 126.7, 126.5, 28.6, 21.4 ppm.

4-(2,5-dioxopyrrolidin-1-yl)benzonitrile (14c): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.7 Hz, 1 H), 7.47 (d, *J* = 8.7 Hz, 1 H), 2.89 (s, 2 H) ppm: ^{13}C NMR (400MHz, $CDCl_3$) δ 175.6, 136.1, 133.4, 133.1, 127.4, 127.1, 118.3, 112.2, 28.6 ppm.

1-(4-nitrophenyl)pyrrolidine-2,5-dione (14d): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, *J* = 1.2 Hz, 2 H), 7.60 (d, *J* = 8.0 Hz, 2 H), 2.96 (s, 4 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.4, 147.2, 137.6, 127.1, 124.6, 28.6 ppm.

2-phenyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (16a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 7.3 Hz, 2 H), 8.27 (d, *J* = 8.3 Hz, 2 H), 7.79 (t, *J* = 8.0 Hz, 2 H), 7.56 (t, *J* = 7.5 Hz, 2 H), 7.49 (t, *J* = 7.4 Hz, 1 H), 7.32 (d, *J* = 7.2 Hz, 2 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.6, 135.7, 134.5, 132.0, 131.8, 129.6, 128.9, 128.9, 128.8, 127.3, 123.0 ppm.

2-*p*-tolyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (16b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 7.3 Hz, 2 H), 8.27 (d, *J* = 8.3 Hz, 2 H), 7.79 (t, *J* = 7.8 Hz, 2 H), 7.36 (d, *J* = 8.1 Hz, 2 H), 7.21 (d, *J* = 8.1 Hz, 2 H), 2.45 (s, 3 H) ppm: ^{13}C NMR (400MHz, $CDCl_3$): 164.6, 139.6, 135.6, 134.5, 132.0, 131.8, 129.8, 129.5, 129.4, 128.7, 127.3, 125.8, 123.1, 21.7 ppm.

4-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)benzonitrile (16c): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 7.3 Hz, 1 H), 8.31 (d, *J* = 8.4 Hz, 1 H), 7.84 (t, *J* = 7.8 Hz 2 H), 7.81 (d, *J* = 8.0 Hz, 1 H), 7.47 (d, *J* = 8.3 Hz, 1 H).

5,5-dimethyl-3-phenylimidazolidine-2,4-dione(18a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (t, *J* = 7.3 Hz, 2 H), 7.35 (d, *J* = 8.3 Hz, 2 H), 7.30 (d, *J* = 7.2 Hz, 1 H), 6.80 (s, 1 H), 1.44 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.6, 156.0, 131.8, 129.3, 128.4, 126.4, 58.9, 25.4 ppm.

5,5-dimethyl-3-*p*-tolylimidazolidine-2,4-dione (18b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (s, 4 H), 7.04 (s, 1 H), 2.38 (s, 3 H), 1.49 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.7, 156.3, 138.5, 130.0, 129.2, 126.4, 58.9, 25.3, 21.4 ppm.

4-(4,4-dimethyl-2,5-dioxoimidazolidin-1-yl)benzonitrile (18c): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.0, 2 H), 7.67 (d, *J* = 8.0, 2 H), 6.72 (s, 1 H), 1.54 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.8, 154.7, 136.1, 133.1, 126.2, 118.4, 111.5, 59.0, 29.9, 25.5 ppm.

5,5-dimethyl-3-(4-nitrophenyl)imidazolidine-2,4-dione (18d): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (dd, *J* = 8.0, 4.0, 2 H), 7.73 (d, *J* = 8.0, 2 H), 7.08 (s, 1 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.8, 154.8, 146.6, 137.8, 126.1, 124.5, 59.0, 25.4 ppm.

3.6 References and Notes:

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Chapter-4: Cu (II) Mediated Formamidation and Amination of Aryl Halides

4.1 Introduction:

Metal catalyzed coupling reactions between aryl halides and amides have been known since long time for their application in academia and pharmaceutical industries.[1] These reactions attract significant attention due to fact that many biologically active compounds possess aryl-nitrogen bond. In fact over 90% of commercially available drugs have at least one nitrogen in their structure.[2] In the past, several research groups reported the Pd catalyzed formation of C-N bond by coupling aromatic halides with amines,[3] amides [4,] and imides [5]. Recently Zhang group reported pd catalyzed amidation of aryl halides using 2-dialkylphosphino-2'-alkoxy-1,1'-

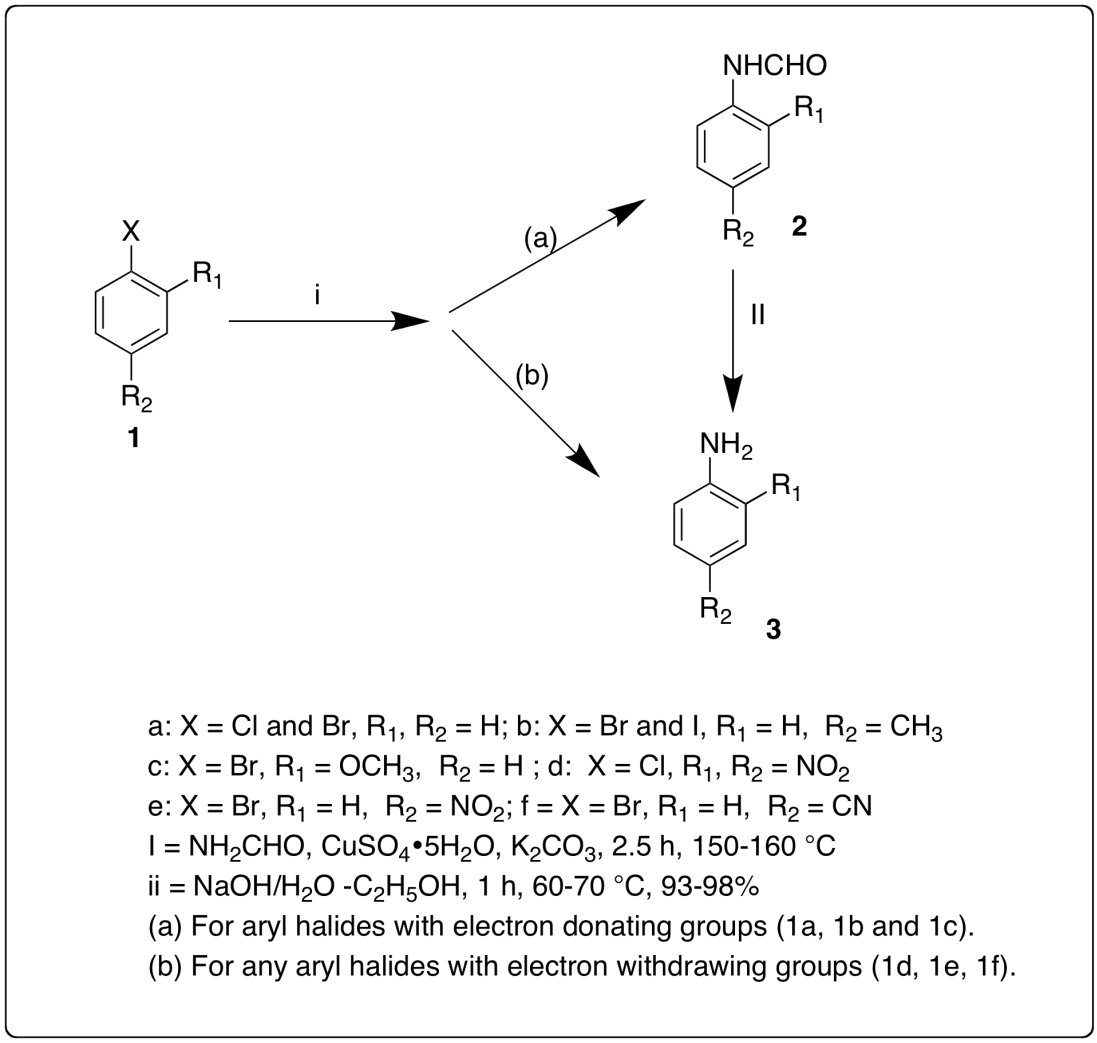
binaphthyl as ligand. [6] In spite of its high cost and use of external ligands it is very hard to apply these reactions in the industrial scale, it is important to mention that removing Pd residues from the reaction media requires special methods, which increase its cost.

The copper catalyzed coupling has emerged as a very good alternate for this, because of its easiness to handle and lower cost and mostly less toxic. In recent years several research groups reported their work on this area mostly by using external ligands such as α-amino acids, [7] bis-pyridylimines, [8] 1,2-diamines, [9] 1,10 – phenanthroline, [10] and 1,3- diketones.[11] However because the use of Cu (I) and external ligands these methods are also relatively expensive and indeed we have to take extra care to handle the reactions. There are also some report regarding of copper catalyzed formamidation of arylboric acids.[12] Therefore it is of paramount interest for both academic and industrial community to develop simple, safe, and economical method for preparation aniline and aryl formamide derivatives from readily available and inexpensive aryl halides. The most economical approach to aryl coupling reactions is to use extremely inexpensive copper (II) sulfate as source of the coupling catalyst.[13]

4.2 Results and Discussion:

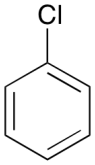
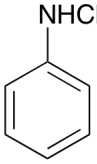
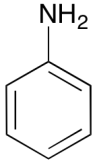
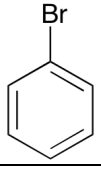
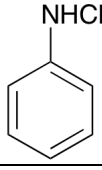
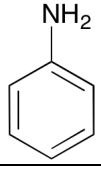
Here we would like to report a simple, efficient, ligand free, and inexpensive method for direct aryl formamidation and amination of readily available aryl halides. Reaction was performed by simple heating formamide suspension of corresponding aryl halide, copper (II) sulfate pentahydrate, and potassium carbonate at 150-160°C for few hours on open air (Figure 1). Product was isolated by extraction of water diluted reaction mixture followed by flash chromatography. Isolated yield are almost quantitative (Table 1).

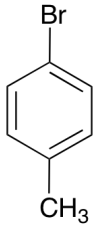
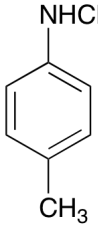
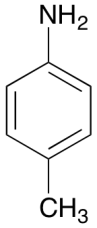
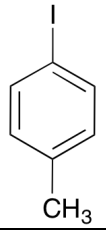
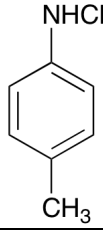
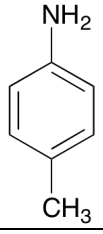
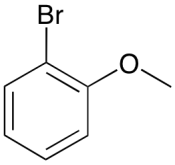
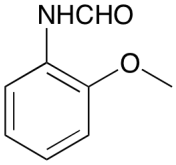
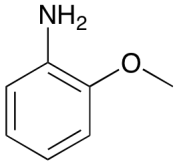
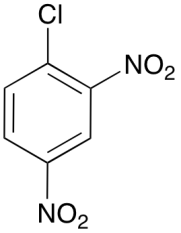
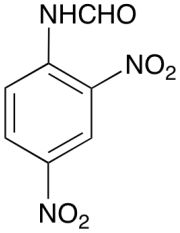
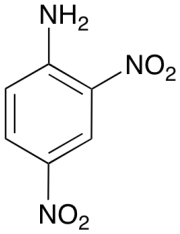
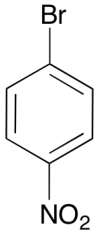
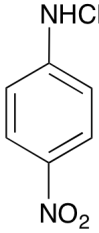
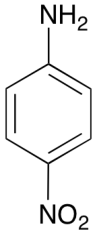
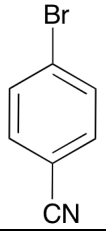
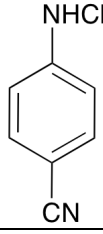
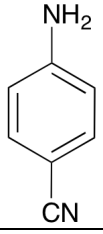
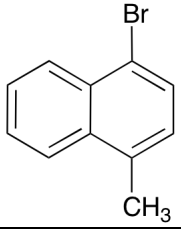
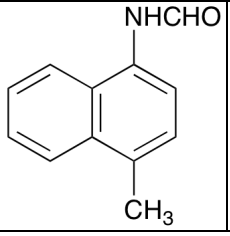
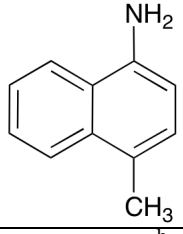
4.2.1 Aryl halides coupling with formamide:



Scheme 4.1 Aryl halides coupling with formamide

Table 4.1 Prepared N-formyl anilines and aniline derivatives

Entry	Aryl Halide	Formamide (2)	Yield 2 (%)	Aniline (3)	Yield 3 (%)
a			93		97[a]
a			95		

b			96		98[a]
b			93		
c			90		96[a]
d			Trace		56[b]
e			Trace		40[b]
f			Trace		40[b]
g			96		97[a]

[a] Prepared in two steps : Formamidation followed by NaOH hydrolysis; [b] Isolated directly from formamidation without NaOH hydrolysis.

The coupling reaction proceeds very well with aryl chlorides, bromides, or iodides. Usually the ideal substrates for coupling reactions are aryl bromides because aryl chlorides are generally less reactive resulting in longer reaction time and lower conversion while aryl iodides are more reactive resulting in formation of undesired byproducts. This makes the purification process somewhat difficult and isolated yields lower, but in our case irrespective of the halogen we ended up with the same result.

Regardless of the nature of the aryl substituents first step of the reaction is always formamidation (path *(a)* products **2a**-**2g**, scheme 3.1). However with electron-withdrawing substituents formed aryl formamide (**2d**, **2e**, and **2f**) are too reactive and they easily hydrolyzed under the formamidation reaction conditions. Consequently only products (**3d**, **3e**, and **3f**) of direct amination were isolated (Table 3.1). On the other hand isolated formaidation products with aryl electron-donating groups were easily hydrolyzed with sodium hydroxide in aqueous ethanol to produce corresponding anilines and in this way aryl amination product can be prepared in the presence ether electron-donating or electron-withdrawing groups.

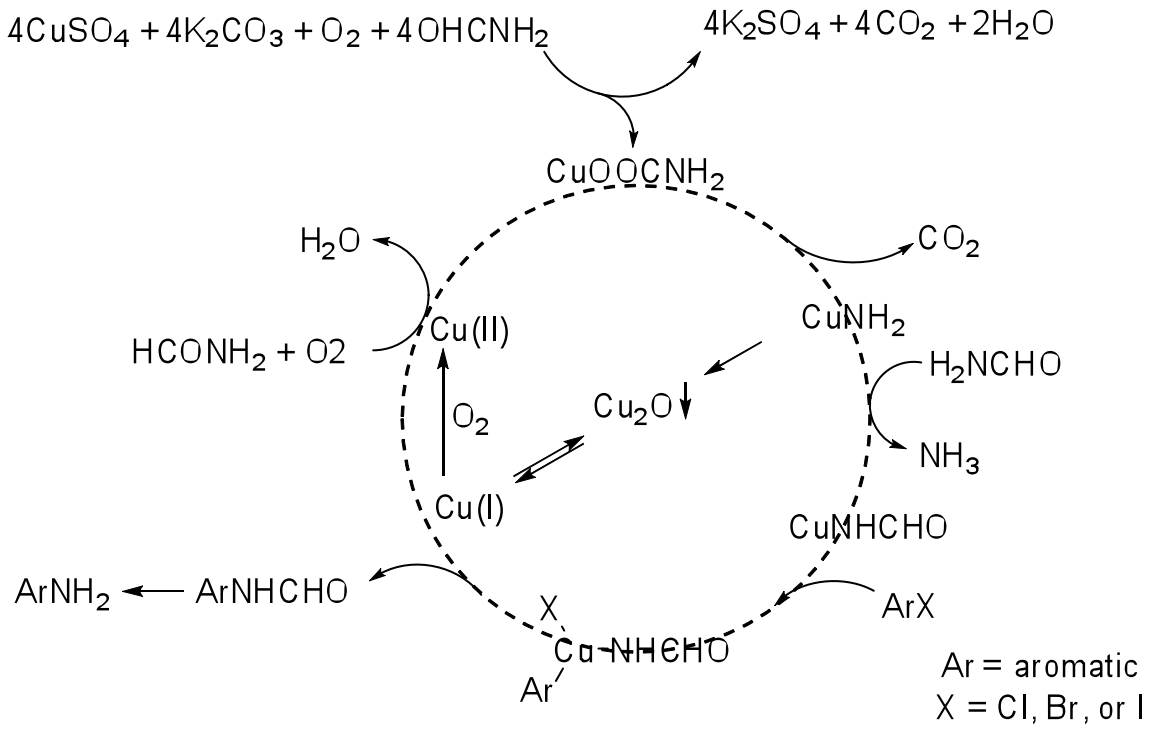
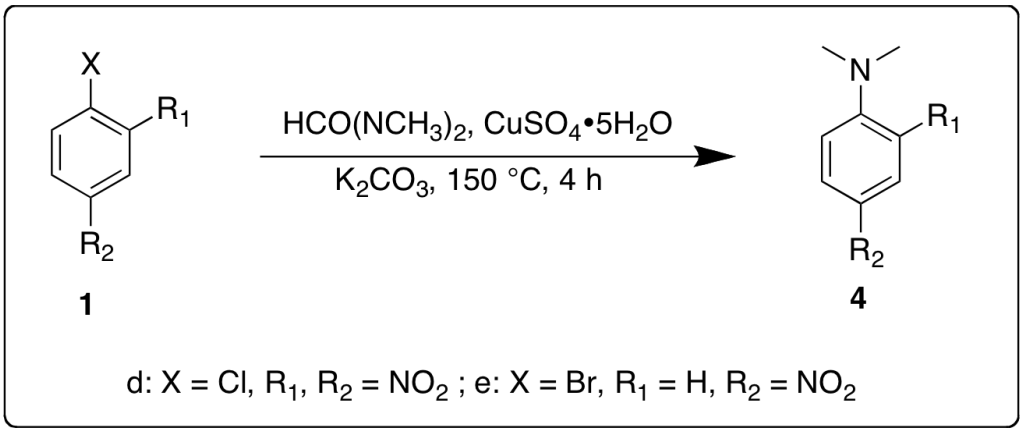


Figure 4.1: Proposed mechanism for $CuSO_4 \bullet 5H_2O$ catalyzed coupling of aryl halides with formamide.

In the nature reaction is transom metal catalyzed aryl coupling[14] between aryl halides and nitrogen nucleophiles such as NHCHO anion. We propose that active coupling reagent is short living cooper (III) complex[15] with aryl and nucleophilic nitrogen moiety (Figure 3.1). During the reaction color of the reaction changes from clear green, to dark blue and finally to dark read suspension. If formamide suspension of cooper (II) sulfate and potassium carbonate is heated at 160°C for few hours formed read precipitate of Cu_2O that can be separated by filtration.[16] If dark red suspension is left stirring overnight on open air colorless solution above the precipitate gradually becomes green (soluble copper II) and eventually all the precipitate (Cu_2O) dissolves to form solution with the original color (copper II). On the bases of these observations we are proposing cooper catalytic cycle for formamidation of aryl halides as outlined in Figure 3.1.

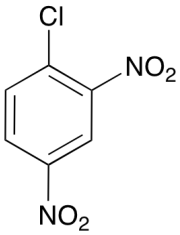
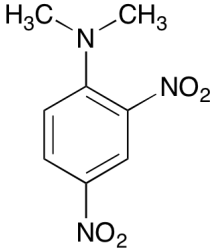
4.2.2 Aryl halides coupling with N,N-dimethylformamide (DMF):

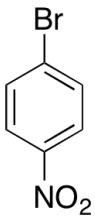
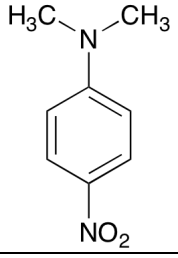
Formamidation with *N*,*N*-dimethylformamide cannot be accomplished because formamide nucleophile cannot be formed during copper (II) mediated reaction, therefore does not come as surprise that there was no reaction with aryl halides that possess electron-donating substituents such as halides **1a**, **1b**, **1c**, **1g**, and **1h**. However when reaction with aryl halides that possess electron-withdrawing substituents such as **1d** and **1e**, and **1f** dimethylamination products were isolated (Scheme 3.2).



Scheme 4.2: Aryl halides coupling with N,N-dimethylformamide (DMF)

Table 4.2: Prepared N,N-dimethyl aniline derivatives

Entry	Aryl Halide	Product (4)	Yield (%)
d			95

e			96

In the course of the reaction red Cu_2O precipitate was formed similar as in the case of reaction with formamide (Figure 3.1). Considering that reaction conditions for both formamide and DMF are identical we proposed similar catalytic mechanism in copper (II) reduction to copper (I) and generating copper (I) dimethylamide (Figure 3.2). In the case of DMF it is not possible to form more stable copper (I) salt as it is the case with formamide (Figure 3.1, CuNHCHO) therefore $CuN(CH_3)_2$ reacts as nucleophile in the classic nucleophilic aromatic substitution (S_NAr)[17] that is feasible for electron deficient aryl halides as it is in our case.

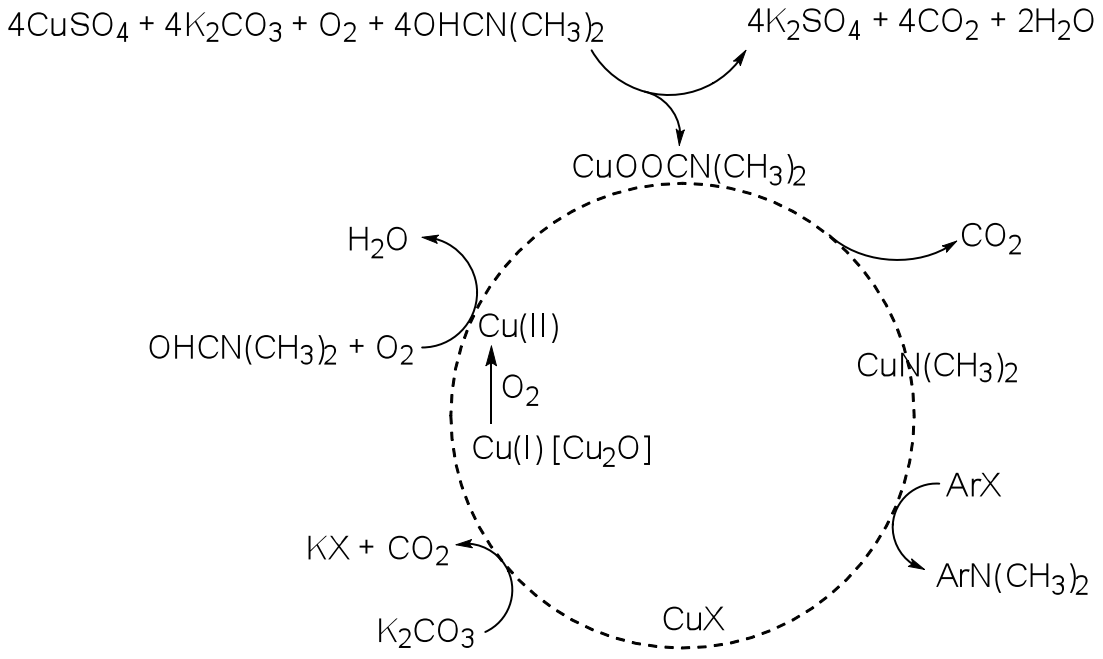


Figure 4.2: Proposed mechanism for $CuSO_4 \bullet 5H_2O$ catalyzed coupling of aryl halides with N,N-dimethyl formamide (DMF).

Up to this moment only results with copper (II) sulfate pentahydrate as the coupling mediator were reported. However reactions with copper (II) chloride, copper (II) acetate as well as with copper (II) oxide were performed. There is no noticeable difference neither in reaction time or isolated yield of the product. Because copper (II) sulfate pentahydrate is broadly available and affordable only the results with this salt were reported here. It is therefore our reasonable assumption that any copper (II) salt with reasonable formamides solubility will be good mediator of formamidation of electron rich aromatic halides.

Initially when prepared are spectroscopically analyzes formamide products always show two sets of signal in both their 1H- as well as 13C-NMRs with rough ratio of 3:1 (Figure 3.3). Considering hydrogen-coupling constant of formamide group (HNCHO) major isomer is *cis* (J = 1.6 Hz) with *trans* (J = 10.8 Hz) only around 30%. To our surprise the ratio of two isomers did not change even when DMSO-d_6 solution was heated with reflux for five minutes. Similar finding were reported in literature.[18] It was estimated that rotation barrier around the C-N formamide bond is around 17 kcal/mol.[19] In fact it was reported that *N*-phenylformamide forms cocrystal with two its isomers.[20]

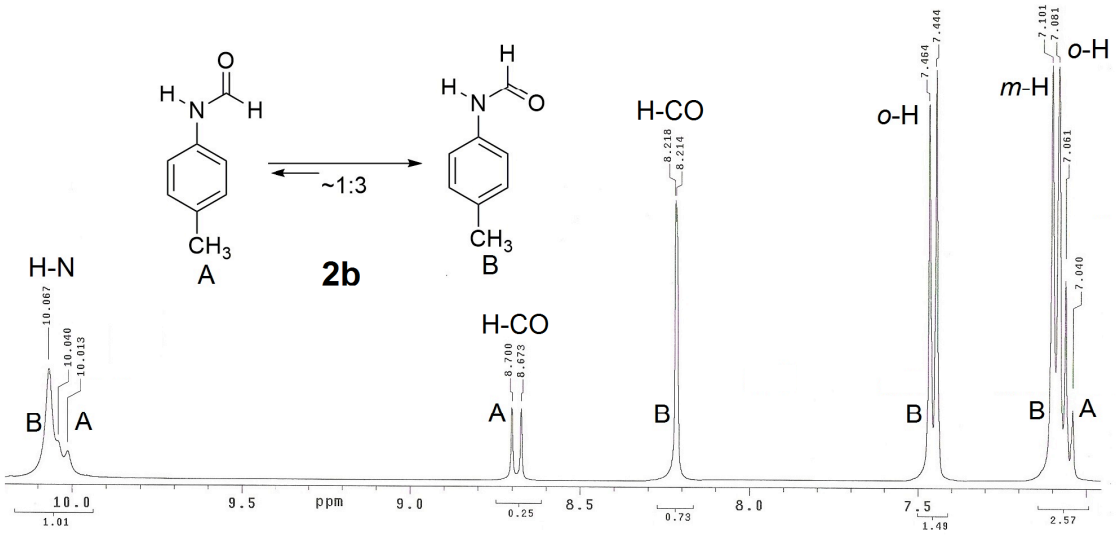


Figure 4.3: ^{1}H NMR showing the *cis* and *trans* isomers of N-*p*-tolylformamide.

4.3 Conclusions:

In conclusion we developed a very good methodology for formamidation and amination of aryl halides. The advantages associated with this methodology such as use of in expensive Cu (II) salts ($CuSO_4 \bullet 5H_2O$, CuO, $CuCl_2 \bullet 2H_2O$, $Cu(OAc)_2 \bullet H_2O$), short reaction times, moderate to excellent yields and finally applicable to broad substrates makes this approach as a versatile method.

4.4 Experimental Section:

General procedure for coupling of aryl halides with formamide: Preparation of *N*-*p*-tolylformamide (2d):

Formamide (10 ml) suspension of 4-bromotolune (**1d**) (342 mg, 2.00 mmol), potassium carbonate (690 mg, 5.00 mmol), and copper (II) sulfate pentahydrate (600 mg; 2.40 mmol) was heated with magnetic stirring at 160°C for 2.5 hours. After cooling reaction mixture was mixed with ice cold water (50 ml) and extracted with dichloromethane (3x20 ml). Combined organic layers were dried over anhydrous sodium sulfate and evaporated to give crude product. This product was further purified by silica gel column chromatography with hexane-ethyl acetate (4:1) as an eluent. Isolated 360 mg (96%) of pure product.

General procedure for hydrolysis of aryl formamides: Preparation of aniline (3a).

Into ethanol (10 ml) solution of *N*-phenylformamide (2b) (120 mg, 1.00 mmol) aqueous (2 ml) sodium hydroxide (40 mg, 1.0 mmol) was added. Resulting solution was refluxed for one hour. Volume of the solvent was reduced (2 ml) and extracted with dichloromethane (3x5 ml). Combined organic extracts were dried over anhydrous sodium sulfate and evaporate to give pure aniline (990 mg; 97%).

General procedure for coupling of aryl halides with formamide: Preparation of *N*-*p*-tolylformamide (4e):

N,N-dimethylformamide (10 ml) suspension of 4-bromonitrobenzene (**1e**) (404 mg, 2,00 mmol), potassium carbonate (690 mg, 5.00 mmol), and copper (II) sulfate pentahydrate (600 mg; 2.4 mmol) was heated with magnetic stirring at 160°C for 2.5 h. After cooling reaction mixture was mixed with ice cold water (50 ml) and extracted with dichloromethane (3x20 ml). Combined organic layers were dried over anhydrous sodium sulfate and evaporated to give crude product.

This product was further purified by silica gel column chromatography with hexane-ethyl acetate (4:1) as an eluent. Isolated 318 mg (96%) of pure product.

4.5 Spectral Section:

***N*-phenylformamide (2a);** ^{1}H NMR ($CDCl_3$)(Complex): δ 9.54 (d, *J* = 10.8 Hz), 9.07 (s), 8.70 (d, *J* = 11.2 Hz), 8.31 (s), 7.60 (d, *J* = 8.0 Hz), 7.30 (q, *J* = 14.8, 7.6 Hz), 7.17-7.09 (m) ppm; ^{13}C NMR ($CDCl_3$): δ 163.7, 160.6, 137.5, 137.3, 130.9, 129.3, 125.5, 125.1, 120.7, 119.0 ppm.

***N*-*p*-tolylformamide (2b);** ^{1}H NMR ($CDCl_3$) (Complex): δ 8.97 (d, *J* = 10.4 Hz), 8.63 (d, *J* = 11.6 Hz), 8.29 (s), 8.20(b), 7.43(d, *J* = 8.4 Hz), 7.11 (q, *J* = 13.6, 8.0 Hz), 6.99 (d, *J* = 8.0 Hz), 2.32 (s), 2.30 (s) ppm; ^{13}C NMR ($CDCl_3$): δ 163.5, 159.8, 135.3, 134.8, 134.6, 134.5, 130.5, 129.8, 120.4, 119.3, 21.1, 21.0 ppm.

***N*-(2-methoxyphenyl)formamide (2c);** ^{1}H NMR ($CDCl_3$) (Complex): δ 8.71(d, *J* = 11.2 Hz), 8.42(s), 8.34(d, *J* = 8.0 Hz), 8.07(b), 7.15(d, *J* = 8.0 Hz), 7.1 (t, *J* = 8.0 Hz), 7.04(t, *J* = 7.6 Hz), 6.92(t, *J* = 8.0 Hz), 6.85(d, *J* = 8.4 Hz), 3.83(s), 3.81(s) ppm; ^{13}C NMR ($CDCl_3$): δ 161.9, 159.8, 149.2, 148.1, 127.6, 126.5, 125.8, 124.9, 121.9, 120.1, 117.9, 111.9, 110.1, 56.1 ppm.

***N*-(4-methylnaphthalen-1-yl)formamide (2g);** ^{1}H NMR ($CDCl_3$)(complex): δ 9.13 (d, *J* = 9.6 Hz), 8.57 (d, *J* = 11.2 Hz), 8.08-7.97 (m), 7.87 (d, *J* = 8 Hz), 7.75 (d, *J* = 8 Hz), 7.62-7.57 (m), 7.54-7.47 (m), 7.27 (t, *J* = 7.6 Hz), 7.19 (d, *J* = 7.2 Hz), 2.70 (s), 2.64 (s) ppm; ^{13}C NMR (CDCL3): 165.0, 160.4, 133.9, 133.5, 133.3, 133.0, 130.9, 129.7, 128.5, 127.5, 126.9, 126.8, 126.5, 126.3, 126.2, 125.1, 125.02 122.4, 121.7, 121.5, 119.6, 19.8, 19.5 ppm.

2,4-dinitroaniline (3e); ^{1}H NMR (dmso); δ 8.79 (s, 1 H), 8.38 (b, 2 H), 8.16 (d, *J* = 7.6 Hz, 1 H), 7.11 (d, *J* = 9.6 Hz, 1 H) ppm; ^{13}C NMR (dmso): 150.5, 135.8, 130.0, 129.4, 124.1, 120.5 ppm.

4-nitroaniline (3e); ^{1}H NMR (dmso): δ 7.93 (d, *J* = 9.2 Hz, 2 H), 6.59 (d, *J* = 9.2 Hz, 2 H), 3.41 (s, 3 H) ppm; ^{13}C NMR (dmso): 156.4, 136.3, 127.1, 113.1 ppm.

4-aminobenzonitrile (3f); ^{1}H NMR ($CDCl_3$): δ 7.41 (d, *J* = 8.0 Hz, 2 H), 6.64 (d, *J*= 8.0 Hz, 2 H), 4.20 (b, 2 H) ppm; ^{13}C NMR (CDCL3): 114.7, 118.0, 134.1, 134.1 ppm.

***N*,*N*-dimethyl-2,4-dinitroaniline (4d);** ^{1}H NMR ($CDCl_3$) : δ 8.56 (s, 1 H), 8.11 (d, *J* = 9.2 Hz, 1 H), 7.00 (d, *J* = 9.6 HZ, 1 H), 3.03 (s, 6 H) ppm; ^{13}C NMR ($CDCl_3$): 149.4, 136.3, 135.6, 127.9, 124.3, 117.0, 42.6 ppm.

***N*,*N*-dimethyl-4-nitroaniline (4e);** ^{1}H NMR ($CDCl_3$): δ 8.10 (d, *J* = 9.2 Hz, 2 H), 6.59 (d, *J* = 9.6 Hz, 2 H), 3.11 (s, 6 H) ppm; ^{13}C NMR ($CDCl_3$); 126.4, 110.4, 40.5 ppm.

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13. Results that are obtained with 98% copper (II) sulfate pentahydrate purchased from Aldrich are also reproduced with 99% copper (II) sulfate pentahydrate from Alpha Chemicals. Current Aldrich price for 100g of $SuSO_4x5H_2O$ is $30.00 whereas 10 pounds 99% $CuSO_4x5H_2O$ from Alpha Chemicals is only $24.

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16. Red precipitate was separated by filtration, washed with water and dried on air. It dissolves in concentrated hydrochloric acid and brown color of the solution quickly changes into green (copper II).

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Chapter -5: Cu(II) Mediated Coupling of Aryl Halides With Cyclic Imides

5.1 Introduction:

N-aryl and alkyl cyclic imides have been known since long time because of their biological activities [(1)] such as anticancer agents [(2)], anticonvulsants [(3)], anti HIV [(4)], Anti inflammatory [(5)], fungicides [(6)], herbicides [(7)], hypolipidemics [(8)], and anti hypertensives [(9)].

5.1.1Imide Derivatives as Anticancer Agents: Amonafide (1) [(10)], a N-alkyl derivative of naphthylimide well known since long time for it's anti cancer activity. Recently it was reported that naphthylimide derivatives inhibits the interaction between Clathrin N-terminal domain (TD) and endocyctic accessory proteins [(11)] (i.e Clathrin inhibition). We also have literature reports of hydantoin derivatives as anti cancer drugs [(12)].

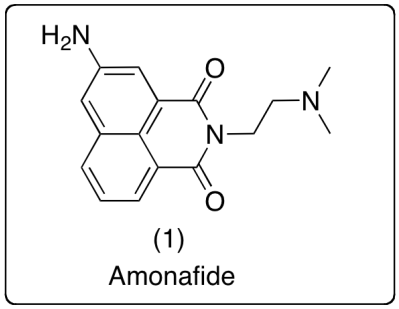


Figure 5.1 Naphthylimide based anticancer drug (Amonafide)

5.1.2 Imide Derivatives as Anticonvulsants:

Epilepsy is one of the most frequent neurological afflictions in man characterized by excessive temporary neuronal discharges resulting in uncontrolled convulsion [(13)]. Anticonvulsants are more accurately called antiepileptic drugs and are sometimes referred to as antiseizure drugs. Although several new anticonvulsants are introduced, some types of seizures are still not

adequately treated with current therapy [14].

Hydantoins [15] and Succinimides [16] a group of cyclic imides have been demonstrated to possess good anticonvulsant property.

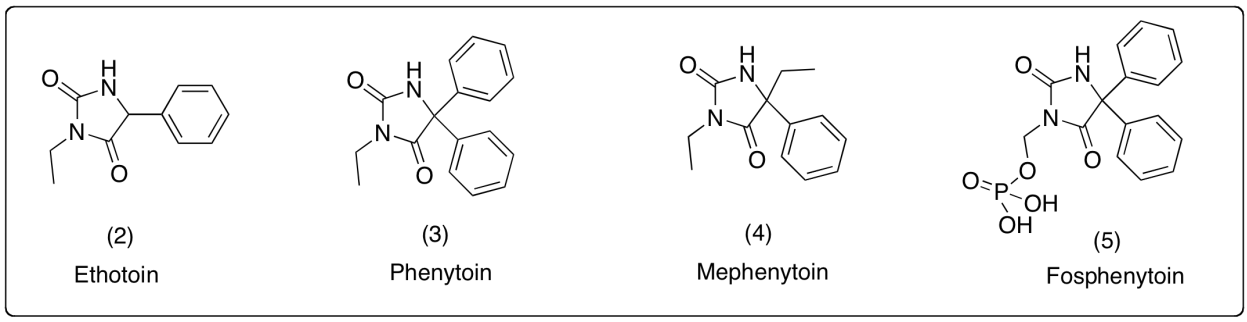


Figure 5.2 Hydantoin based anticonvulsants

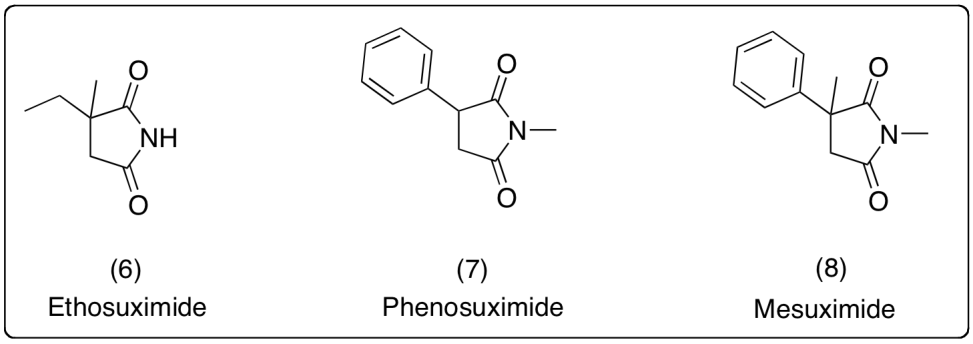


Figure 5.3 Succiniimide based anticonvulsants

5.1.3 Imide Derivatives as Non Steroidal Anti Inflammatory drugs (NSAIDs):

Recently 1,5 – diaryl hydantoin (9) (a cyclic imide) derivatives shown the activity of inhibiting COX-2 enzyme and there by acting as a NSAIDs [17].

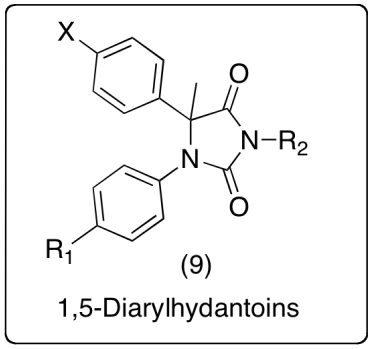


Figure 5.4 General structure of hydantoin based NSAID

5.1.4 Imide derivatives as peptidase inhibitors:

In 1998 Hashimoto group reported that 2-(2,6-diethylphenyl)-l,2,3,4-tetrahydroisoquinoline-l,3-dione (PIQ-22) (10) a N-Aryl cyclic imide as a potent specific inhibitor of amino peptidase N (APN) [18], later on they also reported a library of small molecules which contains a N-phenyl phthalimide skeleton have the capability of inhibiting the dipeptidyl peptidase IV (DPP-IV) [19]. In feb-2013 FDA approved the pomalidomide (3-amino thalidomide) (11) as a treatment for relapsed and refractory multiple myeloma [20]. N-aryl and alkyl succiniimide derivatives are famous for their potential fungicide activity; some of these derivatives can also inhibit the aminopeptidase N [21].

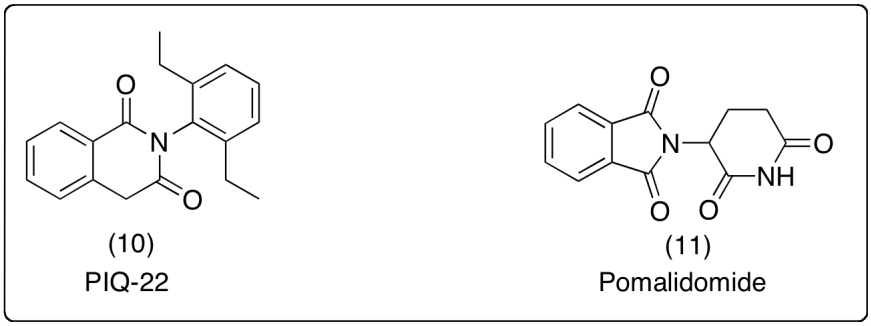


Figure 5.5: Cyclic imide based peptidase inhibitors

Along with their application in pharmaceutical industry, cyclic imide derivatives are well known

for their synthetic utility as intermediates [22] and for their applications in polymer industry [23].

A comprehensive and detailed literature survey reveals that imide derivatives are generally synthesized by the dehydrative condensation of anhydrides with aromatic or aliphatic amines [24], by using ionic liquids [25], Microwave irradiation. [26]

Recently adimurthy et all reported a transmidation process to synthesis the N-substituted phthalimides [27]. However these low yielding condensations frequently requires high temperatures for extended periods of time, additional complications that can include the air sensitivity of many amines as well as the insolubility and hydrolytic susceptibility of the anhydrides [28].

Transition metal catalyzed cross-coupling reactions have revolutionized organic synthesis over the past several decades [29]. There have been a number of recent advances in the field of transition metal promoted C-N bond formation by cross coupling of various N nucleophiles such as amines [30], amides [31], and imides [32] with different aryl and alkyl halides [33] or boronic acids [34] or lead triacetates [35]. So far significant improvements have been achieved in Pd catalyzed C-N cross coupling reactions [36], but it remains hard to apply these reactions to large and industrial scale due to the high cost of Pd and the difficulty in removing Pd residues from polar reaction products [37]. Despite of all these difficulties with Pd, people started employing the Cu catalysis for the cross coupling reactions [38]. Most notably Buchwald and Hartwig published numerous publications on Cu catalyzed C-N bond formation by using various ligands [39]. In 2005, Wsielewski reported a cross coupling of aryl boronic esters with cyclic imides by using $Cu(OAc)_2$ as a catalyst [40], along with these we have various reports, however these are limited since the preparation of highly functionalized substrates usually requires multistep sequences. Because of all these limitations and difficulties we were in a desperate need of a simple end

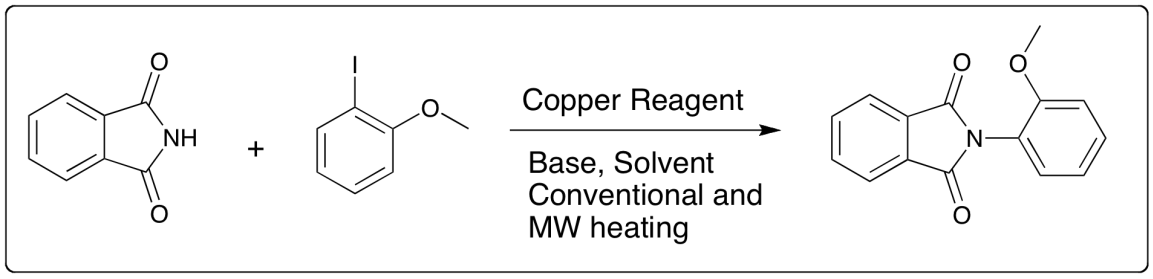
efficient methodology for coupling of cyclic imides with readily available aryl or alkyl halides. We started working on developing a new methodology for coupling cyclic imides with various aryl halides. However, we considered of not using

1) Any Pd/ Pt catalysts,
2) Any external ligands,
3) Any inert (under N_2) and Harsh conditions.

And finally (4) Applicable to diverse substrates.

5.2 Results and discussion:

I started working on coupling of phthalimide with 2-Iodoanisole (Scheme 5.1), the cross coupling reaction required a catalyst, a base and a solvent. So I tried most of the possible combinations by using different bases such as K_2CO_3, KOH, pyridine, 2,4,6-collidine and various solvents like DMF, DMSO, pyridine and water and finally with various copper salts as catalysts (Table 5.1). In most of the cases the reaction ended up with no product formation but in the case of water as a solvent resulted in the formation of copper complex. While in other case when I used the Cu (II) and Cu (I) combination instead of forming the coupled product the reaction ended up with N-methyl phthalimide.

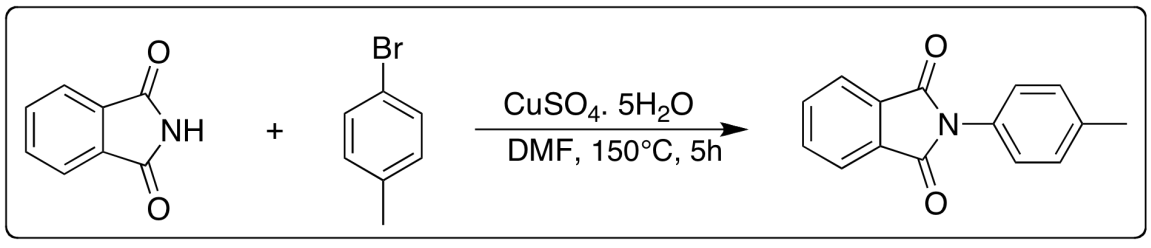


Scheme 5.1 Phthalimide coupling with 2-Iodoanisole:

Table 5.1 Different combinations of base, copper salt and solvent tested on Coupling of phthalimide with 2-Iodoanisole:

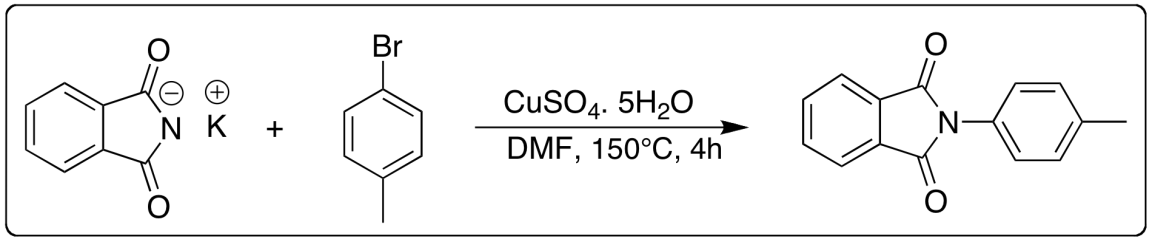
Copper Reagent	Base	Solvent	Result
CuCN	K_2CO_3	DMF	No Reaction
CuCN	K_2CO_3	DMSO	No Reaction
$Cu(OAC)_2$	KOH	DMF	No Reaction
$Cu(OAC)_2$, Cu	K_2CO_3	DMF	No Reaction
$Cu(OAC)_2$	KOH	H2O	Copper Complex
$Cu(OAC)_2$	Pyridine	Pyridine	No Reaction
$Cu(OAC)_2$	2,4,6- Collidine	Pyridine	No Reaction
CuCl	2,4,6- Collidine	Pyridine	No Reaction
$Cu(OAC)_2$, CuCl	K_2CO_3	DMF	N-Methyl Phthalimide
Cu_2O, Cu	K_2CO_3	DMF	No Reaction
Cu_2O	2,4,6- Collidine	Pyridine	No Reaction
Cu_2O	2,4,6- Collidine	Py-HCl	No Reaction

However when I changed the substrate from iodoanisole to p-bromotoluene, and by employing K_2CO_3 as base and $CuSO_4 \bullet 5H_2O$ as the catalyst, the reaction completed in 5 h with the desired coupled product as indicated in scheme 5.2.



Scheme 5.2 phthalimide coupling with *p*-bromotoluene

Later on I changed the simple phthalimide to potassium phthalimide to see the variation in reaction timings (scheme 5.3), and ended up with 4 h required time for reaction completion in the case of potassium phthalimide, which is 1 h short then the required time for simple phthalimide. The optimized reaction conditions required a potassium salt of phthalimide, copper sulfate pentahydrate as a catalyst and DMF as a solvent.



Scheme 5.3 Potassium phthalimide coupling with *p*-bromotoluene.

The progress of the reaction can be monitored visually by color change, which is shown to be in direct correlation with the reaction progress as followed by ^{1}H-NMR spectroscopy (Figure 5.6). The reaction mixture color changes from dark blue to green, yellow, and finally a red suspension in the course of the reaction, as demonstrated in the example of copper (II) sulfated mediated coupling of potassium phthalimide with p-bromotoluene. The reaction is practically completed after four hours of refluxing the DMF suspension.

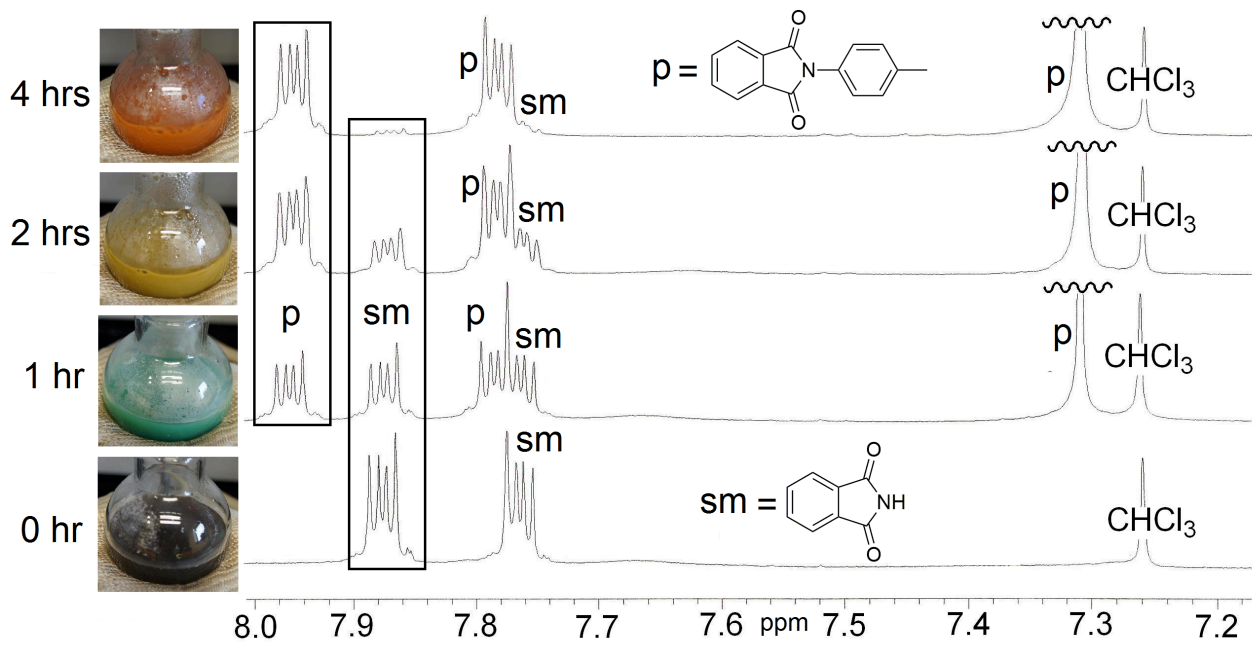
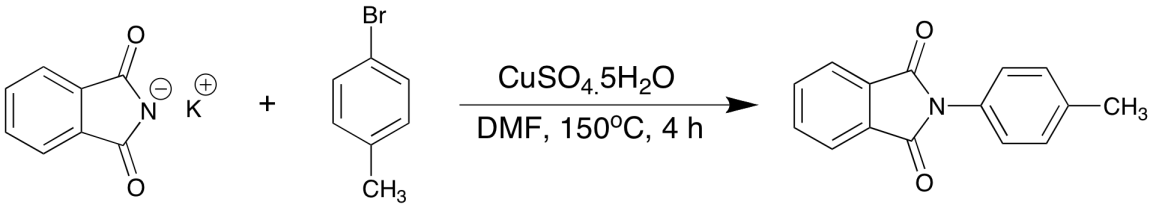


Figure 5.6 Visual and ^{1}H-NMR reaction monitoring of DMF refluxing suspension of potassium phthalimide, *p*-bromotoluene, and copper (II) sulfate pentahydrate

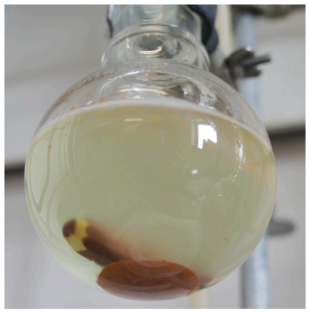


(a) After mixing at room temparature

(b) After refluxing in DMF for 1 h

(c) After refluxing in DMF for 2 h

(d) After refluxing in DMF for 3 h



(e) After refluxing in DMF for 4 h

(f) After refluxing in DMF for 4 h and left at R.T for 10 Min

Figure 5.7 Monitoring the reaction progress by color change

To explore the nature of the copper (II) mediated coupling, the red precipitate (Figure 5.7) was separated by filtration, washed with water and dried. The resulting red powder was not soluble in water but was soluble in concentrated hydrochloric acid, where the solution color quickly changes to green. Same red precipitate was isolated if just suspension of copper (II) sulfate pentahydrate was refluxed for five hours in DMF (Figure 5.8).

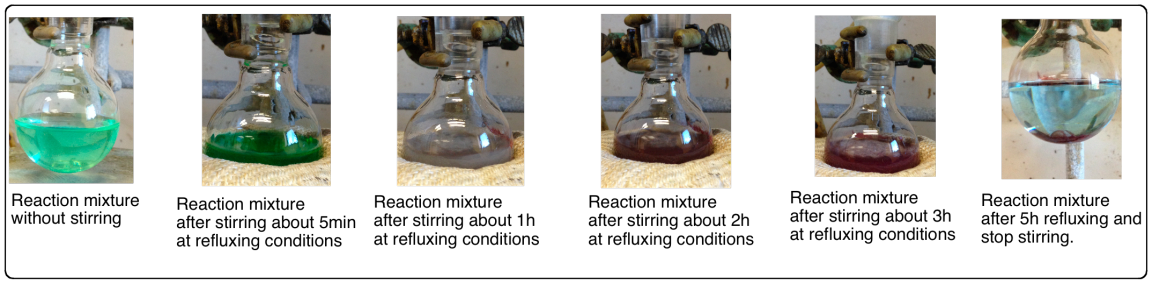


Figure 5.8 Reduction of $CuSO_4.H_2O$ in DMF

However if the DMF suspension of copper (II) sulfate penta hydrate was refluxed with vigorous stirring about overnight with stearic acid, fine small shiny red balls (average diameter around 0.2 mm) were isolated. This material is not soluble in concentrated hydrochloric acid. Therefore in the course of the reaction Cu_2O is formed by reduction of copper (II) with DMF. Because of the low DMF solubility, Cu_2O cannot be further reduced. However copper (I) oxide can be solubilized in the presence of acid, as is the case with stearic acid, where further reduction of copper (I) to copper (0) is possible. Therefore we proposed that the catalytic species for copper mediated coupling is *in situ* generated copper (I). After it is generated copper (I) oxide is removed from the reaction media and is not anymore part of the catalytic cycle (Figure 5.9). This explains the fact that equivalent amounts of copper (II) sulfate pentahydrate are required for the reaction to be completed. There is literature [(12)] evidence for the formation of copper (I) N,N-

dialkylcarbamate [$CuOCON(CH_3)_2$] and our own experiments support the existence of $CuN(CH_3)_2$ in the reaction mixture. For instance when the DMF suspension of equimolar ratio of 4-nitrobromobenzene and copper sulfate pentahydrate was refluxed for eight hours, 4-nitro-*N*,*N*-dimethylaniline was isolated as the only product.

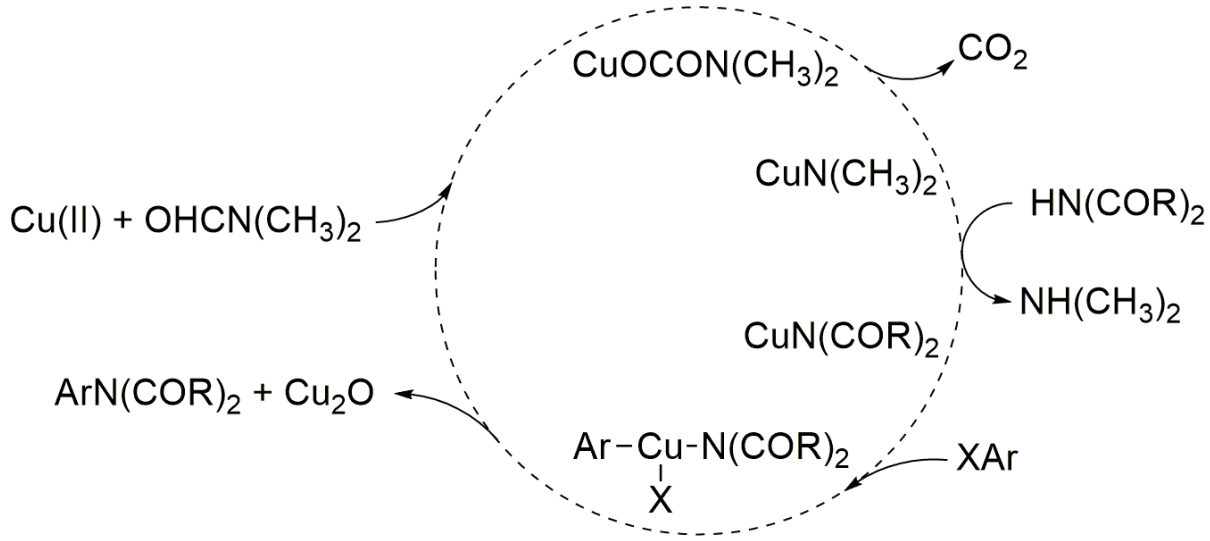


Figure 5.9 Proposed catalytic cycle for coupling of cyclic imides with aryl halides

It is possible to use other sources of copper to perform the studied coupling reactions. We have explored a large number of simple copper (I) and copper (II) salts without using any external ligands. A few selected examples are presented in Figure 5.10. As mentioned, formed Cu_2O precipitated from the reaction mixture and was eliminated from the catalytic cycle. This is fully supported by the finding that there is no product formation with Cu_2O as catalyst after the DMF suspension of the reactants refluxing for four hours. It seems that after the copper (III) salt, $Ar(X)CuN(COR)_2$, rearranges into the product, $ArN(COR)_2$, and copper (I) halide later under the reaction condition precipitates as insoluble copper (I) oxide. On the other hand, the solubility

of copper iodide is slightly higher in comparison to copper (I) oxide and therefore a small amount of catalytic species is present in the solution insuring about a 45% conversion after four hours (Figure 5.10). It is interesting to mention that copper (II) acetate monohydrate is better catalyst than copper (I) iodide but not as good as copper (II) sulfate pentahydrate. It all depends on the solubility of these salts in DMF and in the case of copper (II) salts, the availability for DMF oxidation to $CuOCONMe_2$.

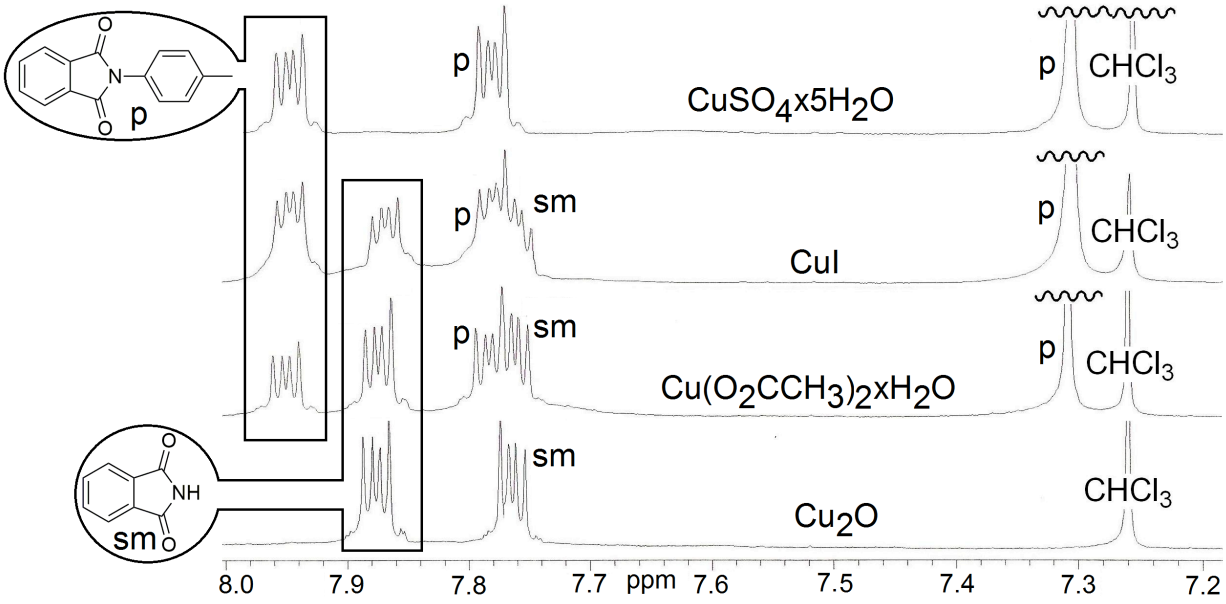


Figure 5.10 Progress of potassium phthalimide reaction with *p*-bromotoluene after four hours of DMF solution refluxing with various Copper Salts.

There are other solvents that can be used as a reaction media for copper mediated reactions. However it is important that the copper (I) catalytic species is formed *in situ* either by copper (II) reduction with solvent or by slow titration with the appropriate reducing reagent. Later, is shown to be complicated due the to rate of addition and/or thermal stability of common reducing reagents such as zinc, ascorbic acid, or glucose. In solvents such as dioxane, and diglyme, the color of copper (II) sulfate changes due to dehydration (Figure 5.11) and after four hours of

refluxing there was neither formation of the product nor copper (II) reduction. However, with cyclohexanol as solvent, after four hours 20% of the conversion was accomplished. The color of the reaction media also indicated the formation of copper (I) oxide. If the cyclohexanol suspension of copper (II) sulfate is refluxed overnight, copper (I) oxide is isolated and the presence of cyclohexanone was confirmed by IR spectroscopy. Therefore cyclohexanol can be used as reaction media but DMF is a superior solvent in respect to reaction time, yields, and method of product isolation.

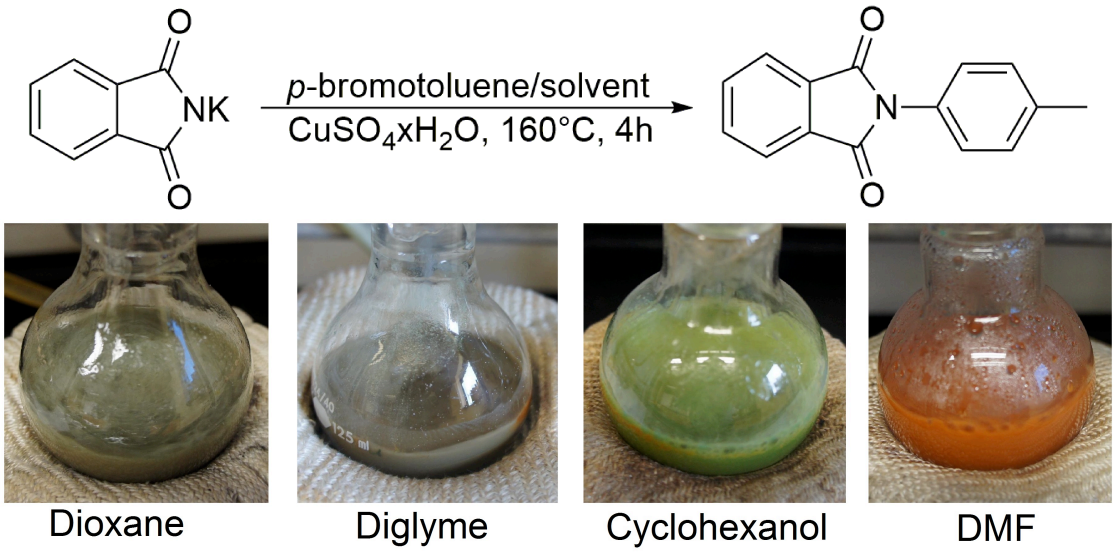
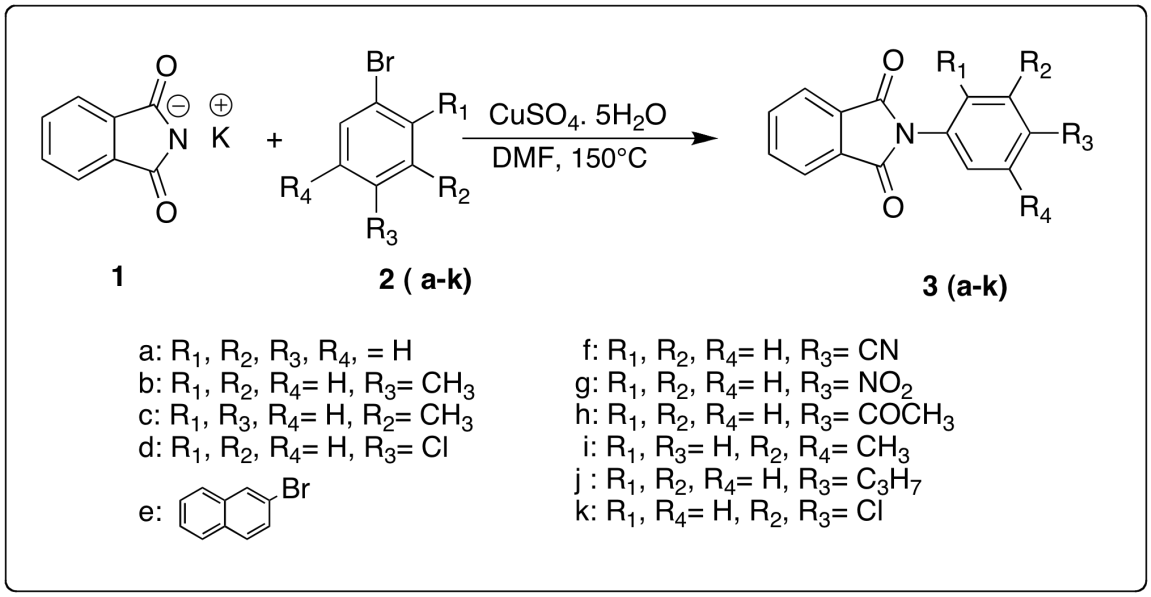


Figure 5.11 The reaction mixture color in various solvents

5.2.1 Potassium phthalimide coupling with aryl halides:

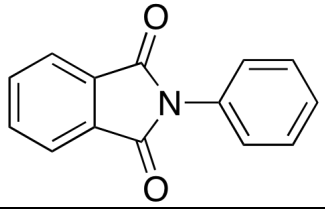
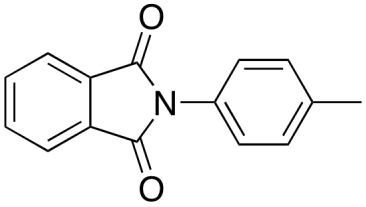
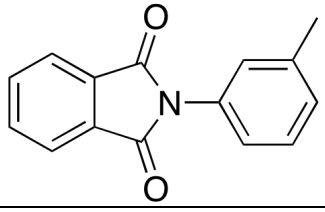
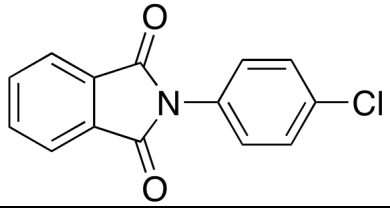
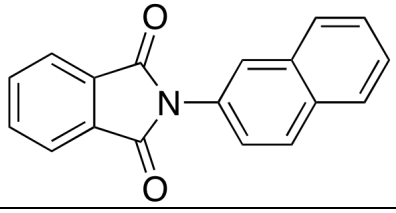
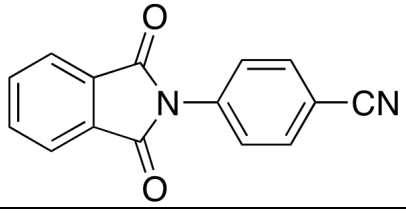
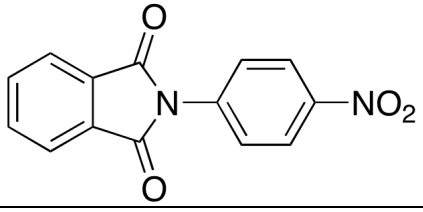
Applying optimized reaction conditions for potassium phthalimide coupling with p-bromotoluene series of N-arylphthalinides has been prepared (Scheme 5.4). The reaction time is less than six hours and isolated yields are almost quantitative except for the preparation of compound 3h. The reaction conditions are ideal for aldol condensation and in the case of the condensation of p-bromoacetophenone with potassium phthalimide, a substantial amount of the

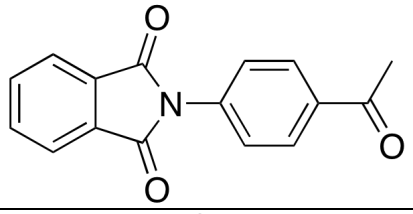
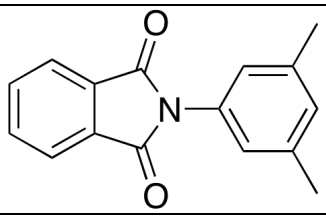
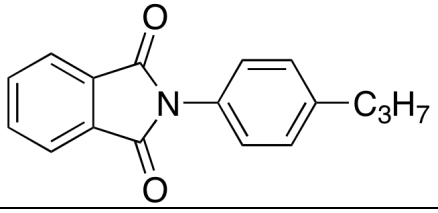
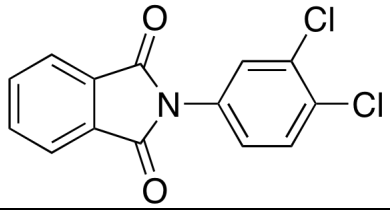
aldol condensate forms as byproduct. Bromo aromatics seem to be and ideal substrates. It is also possible to perform the coupling reaction with chloro aromatics but they seem to be less reactive resulting in longer reaction time. In fact, bromo aromatics can be selectively substituted in the presence chloro aryl compounds as demonstrated in the case of preparation of **3d** and **3j**. The presence of electron withdrawing groups such as nitro and nitrile does not increase isolated yields or shorten reaction time when compare to alkyl (electron donating) substituents. Somewhat lower yields are expected for aryl compounds with acyl substituents, and aryl compounds with formyl (aldehyde) groups are oxidized, such as the case in preparation of **3l** from potassium phthalimide and p-bromobenzaldehyde.



Scheme 5.4 Potassium phthalimide coupling with aromatic halides

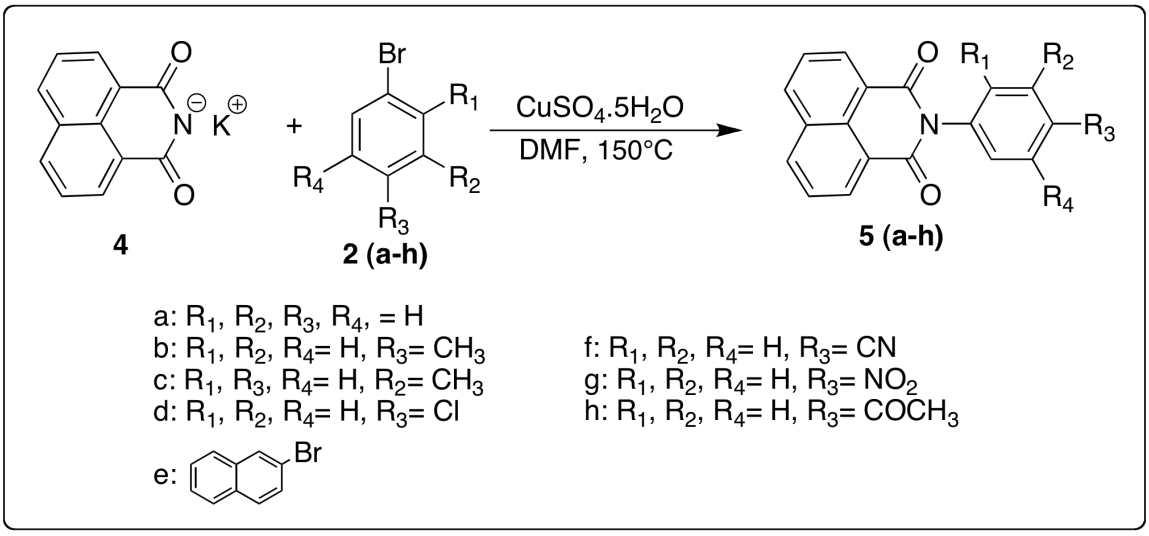
Table 5.2 Prepared N-Arylphthalimides

Entry #	Product Structure	Reaction Time (h)	% Yield
3a		4	96
3b		4	94
3c		6	93
3d		6	95
3e		2.5	89
3f		4	95
3g		5	92

3h		3	62
3i		2.5	95
3j		2.5	95
3k		3	93

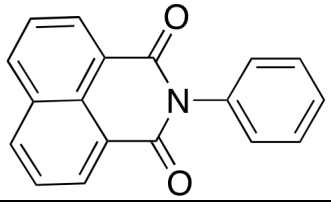
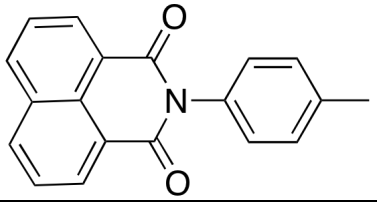
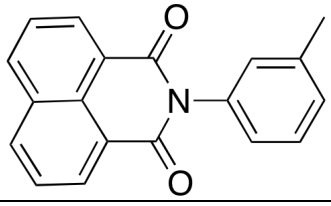
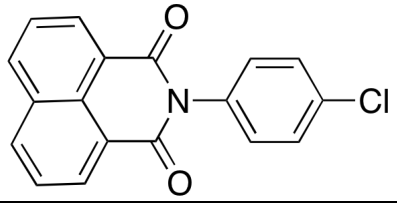
5.2.2 Potassium 1,8-naphthalimide coupling with aryl halides:

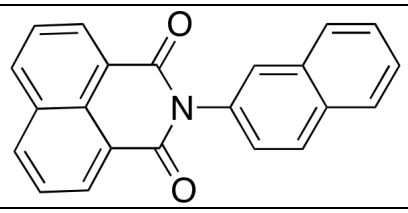
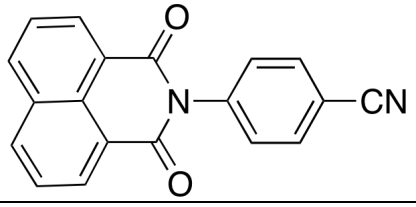
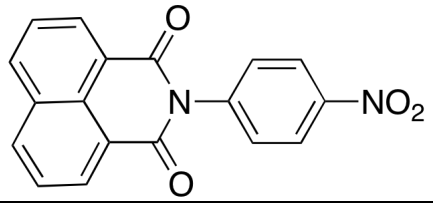
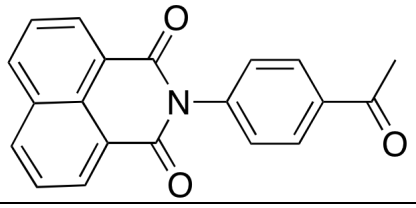
Benzoisoquinoline-1,3-diones attracted some interest because of their antitumor and antimicrobial activities. The majority of these compounds studied up to this point are N-alkyl substituted. [15] It was of our interest to develop a simple methodology for the preparation of a library of N-arylbenzoisoquinoline-1,3-diones (Scheme 5.5). The procedure for preparation of these compounds is similar to the preparation of phthalimides, with only one difference that these materials have in general lower solubility. Isolated yields are almost quantitative.



Scheme 5.5 Potassium-1,8-naphthalimide coupling with aromatic halides.

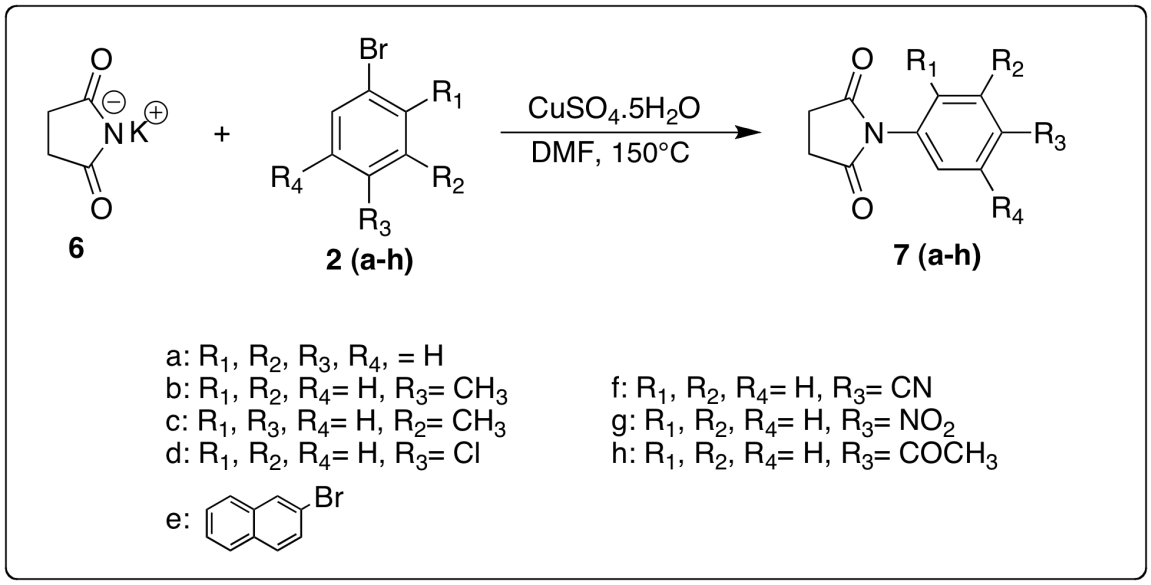
Table 5.3 Prepared N-Aryl-1,8-naphthalimides

Entry #	Product Structure	Reaction Time (h)	% Yield
5a		5	94
5b		5	96
5c		5	91
5d		4	94

5f		6	89
5f		4	93
5g		3	91
5h		5	53

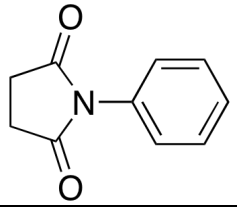
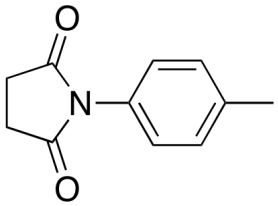
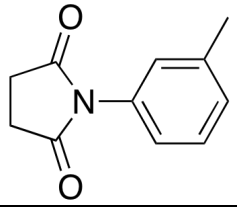
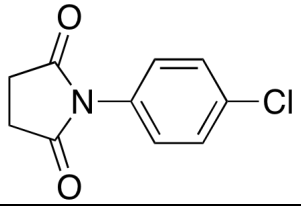
5.2.3 Potassium succinimide coupling with aryl halides:

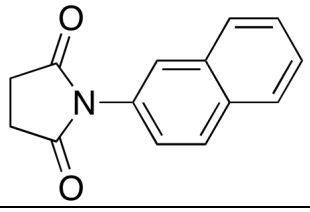
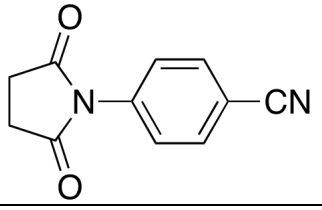
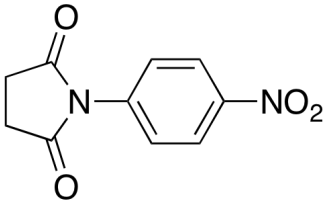
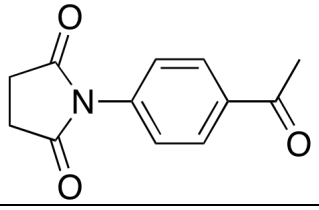
Five membered nitrogen contain ring with carbonyl groups are a common motif in many natural products [16] and materials with interesting biological activity [17]. Our synthetic procedure offers a new route to prepare a structurally diverse library of these valuable compounds with direct attachment of the aryl moiety nitrogen of 2,5-pyrrolidinedione as demonstrated in Scheme 5.6.



Scheme 5.6 Potassium succinimide coupling with aromatic halides.

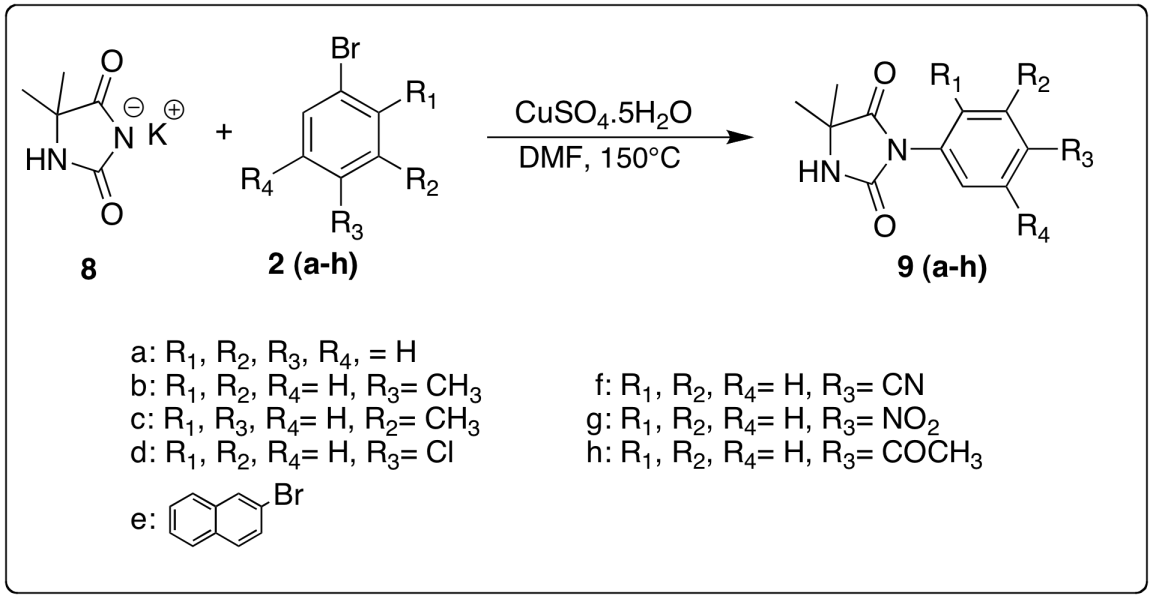
Table 5.4 Prepared N-arylsuccinimides

Entry #	Product Structure	Reaction Time (h)	% Yield
7a		5	92
7b		5	96
7c		5	94
7d		4	91

7e		6	89
7f		4	95
7g		3	67
7h		5	91

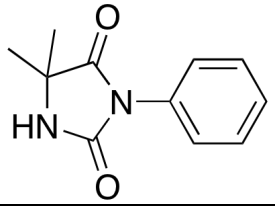
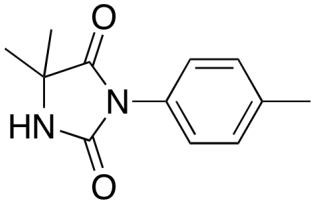
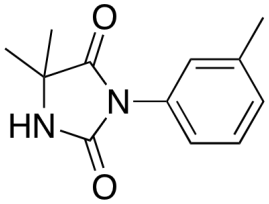
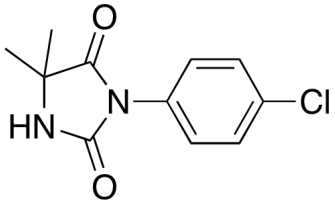
5.2.4 Potassium 5,5-dimethyl hydantoin coupling with aryl halides:

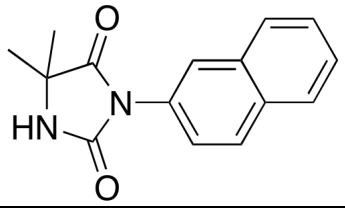
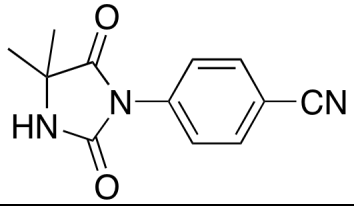
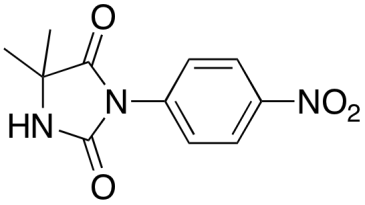
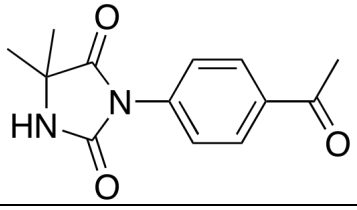
The final group of interesting compounds that can be prepared by utilizing our synthetic approach are the 3- aryl hydantoins. Besides classical application of hydantoin derivatives as anticonvulsants [(18)] there is a recent renaissance of application of 3-aryl hydantoins in modern medicinal chemistry [(19)]. This warrants for a new short and efficient method for the preparation of 3-aryl hydantoin from readily available hydantoins that have an available 3-position for arylation. As demonstrated in Scheme 5.7, our synthetic procedure can be used for the preparation of a diverse library of 3-aryl hydantoins, starting from 3-unsubstituted hydantoins.



Scheme 5.7 Potassium-5,5-Dimethyl hydantoin coupling with aromatic halides

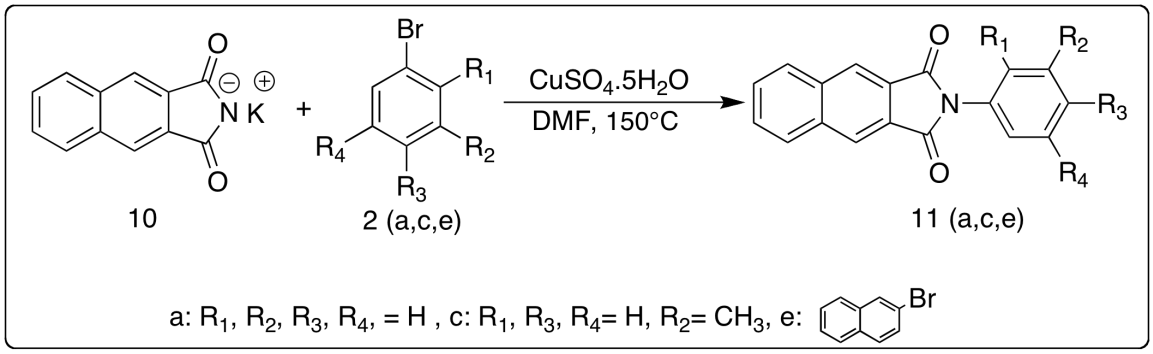
Table 5.5 Prepared N-aryl-5,5-dimethyl hydantoins

Entry #	Product Structure	Reaction Time (h)	% Yield
9a		3	94
9b		2	95
9c		3	96
9d		2	92

9e		4	91
9f		2	93
9g		2	64
9h		3	90

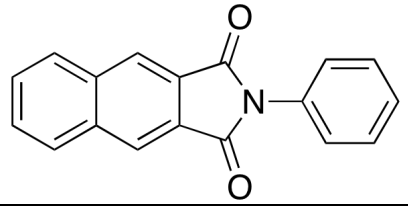
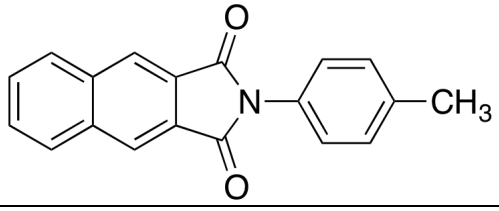
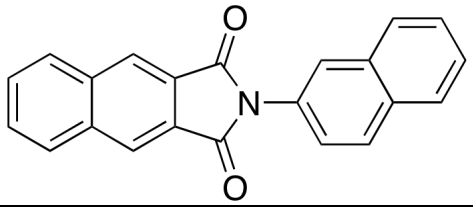
5.2.5 Potassium 1,3-naphthalimide coupling with aryl halides:

Finally we also explored our developed methodology with 2,3-Naphthylimide. However the methodology worked with only few substrates (Scheme 5.8).



Scheme 5.8 Potassium-2,3-Naphthalimide coupling with aromatic halides.

Table 5.6 Prepared N-aryl-2,3-naphthalimides

Entry #	Product Structure	Reaction Time (h)	Yield (%)
11a		5	86
11c		5	84
11e		6	76

5.3 Conclusions:

In conclusion we developed a decent methodology for the preparation of N-aryl cyclic imides by coupling aryl halides with various cyclic imides such as phthalimide, 1,8-naphthalimide, succinimide, 5,5-dimethyl hydantoin and 2,3 naphthalimide. The coupling reaction was screened with various copper salts like $CuSO_4 \bullet 5H_2O$, CuO, CuI, $Cu(OAc)_2 \bullet H_2O$, and found that $CuSO_4 \bullet 5H_2O$ is the ideal catalyst. We also found that the DMF is the ideal solvent. The mechanistic investigations tells us the requirement of equivalent amount of catalyst. The highlights of the reactions are (1) Use of inexpensive Cu (II) salt (2) Monitoring the reaction progress by color change (3) Relatively short reaction times (4) Applicability to diverse substrates and (5) Moderate to excellent yields.

5.4 Experimental and Spectral section:

Preparation of imides from anhydrides;

1,8 Naphthalimide: 1,8 Nahthalic anhydride (5000 mg, 25.00 mmol), 3.28 ml of 29% aqueous ammonia (2900 mg, 50.00 mmol), and water (15 ml) were mixed to form yellow slurry. The yellow slurry was heated with stirring to 70°C, then held at that temperature for about 2 h. The mixture was then cooled to room temperature and filtered. The product was then washed with 75 to 100 ml of water until the pH of the washed water was neutral. The product, 1,8-naphhthalimide (4720 mg, 95%) was isolated as a powder, upon drying and was used directly in the next step.

2,3 Naphthalimide: 2,3 Naphthalic anhydride (5000 mg, 25.00 mmol), 3.28 ml of 29% aqueous aamonia (2900 mg, 50.00 mmol) and water (15 ml) were mixed to form a slurry. The slurry was heated with stirring to 70°C, then held at that temperature for about 2 h. The mixture was then cooled to room temperature and filtered. The product was then washed with 75 to 100 ml of water until the pH of the washed water was neutral. The product 2,3-Naphthalimide (4436 mg, 90%) was isolated as a powder, upon drying and was used directly in the next step.

Preparation of potassium salts of imides:

Potassium Phthalimide (1): Phthalimide (1470 mg, 10.00 mmol) was dissolved in 30 ml of hot absolute ethanol and to this hot solution was added potassium hydroxide (560 mg, 10.0 mmol) dissolved in 4 ml of 75% alcohol. The final solution was cooled at once and the Potassium Phthalimide that precipitates was filtered and washed with ice-cold ethanol, dried and used for further reactions.

Potassium 1,8-Naphthalimide (4): 1,8-Naphthalimide (1970 mg, 10.00 mmol) was dissolved in 30 ml of hot absolute ethanol and to this hot solution was added potassium hydroxide (560 mg, 10.0 mmol) dissolved in 4 ml of 75% alcohol. The final solution was cooled at once and the Potassium Phthalimide that precipitates was filtered and washed with ice-cold ethanol, dried and used for further reactions.

Potassium Succinimide (6): Succinimide (990 mg, 10.0 mmol) was dissolved in 30 ml of hot absolute ethanol and to this hot solution was added potassium hydroxide (560 mg, 10.0 mmol) dissolved in 4 ml of 75% alcohol. The final solution was cooled at once and the Potassium Phthalimide that precipitates was filtered and washed with ice-cold ethanol, dried and used for further reactions.

Potassium 5,5-Dimethylhydantoin (8): 5,5-Dimethylhydantoin (1280 mg, 10.00 mmol) was dissolved in 30 ml of hot absolute ethanol and to this hot solution was added potassium hydroxide (560 mg, 10.0 mmol) dissolved in 4 ml of 75% alcohol. The final solution was cooled at once and the Potassium Phthalimide that precipitates was filtered and washed with ice-cold ethanol, dried and used for further reactions.

Potassium 2,3-Naphthalimide (10): 1,8-Naphthalimide (1970 mg, 10.00 mmol) was dissolved in 30 ml of hot absolute ethanol and to this hot solution was added potassium hydroxide (560 mg, 10.0 mmol) dissolved in 4 ml of 75% alcohol. The final solution was cooled at once and the Potassium Phthalimide that precipitates was filtered and washed with ice-cold ethanol, dried and used for further reactions.

Preparation of Coupled Products along with spectral data

Preparation of 2-phenylisoindoline-1, 3-dione (3a): To a DMF suspension of Potassium Phthalimide (222 mg, 1.20 mmol) was added Bromo Benzene (157mg, 1.00 mmol) and $CuSO_4.5H_2O$ (250 mg, 1.00 mmol). The reaction mixture was heated at 150°C for 4 h and monitored the completion reaction by TLC. The reaction mixture initially blue in color changes to green, then in to brown red, leaving residual cuprous oxide. After the reaction is complete the reaction mixture was cooled to room temperature and poured on to ice. The precipitate obtained was washed with 10% HCl solution, dried and washed over a bed of silica with dichloromethane to obtain pure product (214 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.79 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.50 (t, *J* = 7.3 Hz, 2 H), 7.44 (dt, *J* = 7.6, 1.4 Hz, 1 H), 7.40 (dt, *J* = 2.7, 1.6 Hz, 1 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.5, 134.6, 132.0, 131.9, 129.3, 128.33, 126.8, 124.0 ppm.

2-*p*-tolylisoindoline-1,3-dione (3b): Title compound was prepared according to general method described above using *p*-bromo toluene and potassium phthalimide. The product was purified by running on a bed of silica with dichloromethane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 5.4, 3.1 Hz, 2 H), 7.77 (dd, *J* = 5.4, 3.1 Hz, 2 H), 7.31 (s, 4 H), 2.41 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.7, 138.4, 134.6, 132.0, 130.2, 130.0, 129.9, 129.24, 126.9, 126.7, 126.6, 123.9, 21.5 ppm.

2-*m*-tolylisoindoline-1,3-dione (3c): Title compound was prepared according to general method described above using *m*-bromo toluene and potassium phthalimide. The product was purified by running on a bed of silica with dichloromethane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 5.4, 3.1 Hz, 1 H), 7.79 (dd, *J* = 5.4, 3.1 Hz, 1 H), 7.39 (t, *J* = 7.8 Hz, 1 H),

7.22 (d, J = 7.8 Hz, 2 H), 2.42 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.6, 139.4, 134.6, 132.0, 131.7, 129.3, 129.2, 127.5, 124.0, 124.0, 21.6.

2-(3,5-dimethylphenyl)isoindoline-1,3-dione (3i): Title compound was prepared according to general method described above using 5-bromo *m*-xylene and potassium phthalimide. The product was purified by running on a bed of silica with dichloromethane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (dd, J = 5.4, 3.1 Hz, 2 H), 7.78 (dd, J = 5.4, 3.1 Hz, 2 H), 7.05 (s, 1 H), 7.01 (s, 2 H), 2.37 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.7, 139.1, 134.5, 132.1, 131.6, 130.4, 124.7, 123.9, 21.5 ppm.

2-(4-propylphenyl)isoindoline-1,3-dione (3j): Title compound was prepared according to general method described above using 1-bromo 4-propyl benzene and potassium phthalimide. The product was purified by running on a bed of silica with dichloromethane to obtain a yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, J = 5.4, 3.1 Hz, 2 H), 7.78 (dd, J = 5.4, 3.1 Hz, 2 H), 7.37 - 7.28 (m, 4 H), 2.64 (t, J =7.6 Hz, 2 H), 1.67 (sextet, J =7.2Hz, 2 H), 0.98 (t, J = 7.2 Hz, 3 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.7, 143.1, 134.6, 132.1, 129.4, 126.6, 123.9, 38.0, 24.7, 14.1 ppm.

2-(4-chlorophenyl)isoindoline-1,3-dione (3d): Title compound was prepared according to general method described above using 1-bromo 4-chloro benzene and potassium phthalimide. The product was purified by running on a bed of silica with dichloromethane to obtain a pale yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97(dd, J = 5.4, 3.1 Hz, 2 H), 7.81 (dd, J = 5.4, 3.1 Hz, 2 H), 7.48 (d, J = 8.8 Hz, 2 H), 7.42 (d, J = 8.8 Hz, 2 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.2, 134.8, 134.0, 132.5, 131.8, 130.4, 129.5, 128.2, 127.9, 124.1 ppm.

2-(naphthalen-2-yl)isoindoline-1,3-dione (3e): Title compound was prepared according to general method described above using 2-bromo naphthalene and potassium phthalimide. The product was purified by running on a bed of silica with dichloromethane to obtain a pale yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 3.1 Hz, 1 H), 7.99 - 7.96 (m, 2 H), 7.95 (d, *J* = 1.8 Hz, 1 H), 7.90 (dd, *J* = 5.9, 3.5 Hz, 2 H), 7.82 (dd, *J* = 5.9, 3.5 Hz, 2 H), 7.57 - 7.52 (m, 3 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.7, 134.7, 133.5, 132.8, 132.0, 129.2, 128.5, 128.0, 126.9, 126.8, 125.8, 124.4, 124.0 ppm.

2-(3,4-dichlorophenyl)isoindoline-1,3-dione (3k): Title compound was prepared according to general method described above using 4-bromo-1,2-dichlorobenzene and potassium phthalimide. The product was purified by running on a bed of silica with dichloromethane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.82 (dd, *J* = 5.5, 3.1 Hz, 2 H), 7.64 (d, *J* = 2.4 Hz, 1 H), 7.58 (d, *J* = 8.6 Hz, 1 H), 7.37 (dd, *J* = 8.6, 2.4 Hz, 1 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.9, 135.0, 133.2, 132.3, 131.7, 131.3, 131.0 128.4, 125.8, 124.2 ppm.

4-(1,3-dioxoisoindolin-2-yl)benzonitrile (3f): Title compound was prepared according to general method described above using 4-bromo benzonitrile and potassium phthalimide. The product was purified by running on a bed of silica with dichloromethane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (dd, *J* = 5.5, 3.1 Hz, 2 H), 7.84 (dd, *J* = 5.5, 3.1 Hz, 2 H), 7.80 (d, *J* = 8.7 Hz, 2 H), 7.69 (d, *J* = 8.7 Hz, 2 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.7, 136.2, 135.2, 133.2, 131.6, 126.7, 124.3, 118.5, 111.5 ppm.

2-(4-nitrophenyl)isoindoline-1,3-dione (3g): Title compound was prepared according to general method described above using 1-bromo 4-nitro benzene and potassium phthalimide. The

product was purified by running on a bed of silica with dichloromethane to obtain a yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 9.2 Hz, 2 H), 8.00 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.85 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.77 (d, *J* = 9.2 Hz, 2 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.6, 146.6, 137.8, 135.2, 131.6, 126.6, 124.7, 124.4 ppm.

2-(4-acetylphenyl)isoindoline-1,3-dione (3h): Title compound was prepared according to general method described above using 4- bromo acetophenone and potassium phthalimide. The product was purified by running on a bed of silica with 20% ethyl acetate, hexane to obtain a yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.6 Hz, 2 H), 7.92 (dd, *J* = 5.4, 3.1 Hz, 3 H), 7.83 (dd, *J* = 5.4, 3.1 Hz, 2 H), 7.63 (d, *J* = 8.6 Hz, 2 H), 2.65 (s, 3 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 197.3, 167.0, 136.3, 136.2, 135.0, 131.8, 129.4, 126.3, 124.2, 26.9 ppm.

Preparation of 2-phenyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5a): To a DMF suspension of Potassium salt of 1,8 Naphthalimide (282 mg, 1.20 mmol) was added bromobenzene (157 mg, 1.00 mmol) and $CuSO_4.5H_2O$ (250 mg, 1.00 mmol). The reaction mixture was heated at 150°C for 5 h and monitored the completion reaction by TLC. The reaction mixture initially blue in color changes to green, then in to brown red, leaving residual copper. After the reaction is complete the reaction mixture was cooled to room temperature and poured on to ice. The precipitate obtained was washed with 10% HCl Solution, dried and run over a bed of silica to obtain pure product (256 mg, 94%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 7.3 Hz, 2 H), 8.27 (d, *J* = 8.3 Hz, 2 H), 7.79 (t, *J* = 8.0 Hz, 2 H), 7.56 (t, *J* = 7.5 Hz, 2 H), 7.49 (t, *J* = 7.4 Hz, 1 H), 7.32 (d, *J* = 7.2 Hz, 2 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.6, 135.7, 134.5, 132.0, 131.8, 129.6, 128.9, 128.9, 128.8, 127.3, 123.0 ppm.

2-*p*-tolyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5b): Title compound was prepared according to general method described above using *p*-bromo toluene and potassium 1,3-dioxo-1,3-dihydrobenzo[*de*]isoquinolin-2-ide.. The product was purified by running on a bed of silica with 5% ethyl acetate, hexane to obtain a pale yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 7.3 Hz, 2 H), 8.27 (d, *J* = 8.3 Hz, 2 H), 7.79 (t, *J* = 7.8 Hz, 2 H), 7.36 (d, *J* = 8.1 Hz, 2 H), 7.21 (d, *J* = 8.1 Hz, 2 H), 2.45 (s, 3 H) ppm: ^{13}C NMR (400MHz, $CDCl_3$): 164.6, 139.6, 135.6, 134.5, 132.0, 131.8, 129.8, 129.5, 129.4, 128.7, 127.3, 125.8, 123.1, 21.7 ppm.

2-*m*-tolyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5c): Title compound was prepared according to general method described above using *m*-bromo toluene and potassium 1,3-dioxo-1,3-dihydrobenzo[*de*]isoquinolin-2-ide.. The product was purified by running on a bed of silica with 5% ethyl acetate, hexane to obtain a pale yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 7.3 Hz, 2 H), 8.25 (d, *J* = 8.1 Hz, 2 H), 7.77 (t, *J* = 7.8 Hz, 2 H), 7.45 (t, *J* = 7.6 Hz, 1 H), 7.30 (d, *J* = 7.5 Hz, 1 H), 7.14 (d, *J* = 8.4 Hz, 2 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.7, 138.8, 134.4, 133.0, 132.0, 131.8, 130.4, 128.8, 128.5, 127.2, 123.1, 21.6 ppm.

2-(4-chlorophenyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5d): Title compound was prepared according to general method described above using 1-bromo 4-chloro benzene and potassium 1,3-dioxo-1,3-dihydrobenzo[*de*]isoquinolin-2-ide.. The product was purified by running on a bed of silica with 5% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 7.3 Hz, 2 H), 8.29 (d, *J* = 8.3 Hz, 2 H), 7.80 (t, *J* = 7.8 Hz, 2 H), 7.49 (d, *J*=7.6 Hz, 2 H), 7.27 (d, *J* = 7.6 Hz, 2 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.4, 134.8, 134.7, 134.1, 132.0, 130.7, 130.3, 129.9, 128.7, 127.3, 122.8 ppm.

2-(naphthalen-2-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5e): Title compound was prepared according to general method described above using 2-bromo naphthalene and potassium 1,3-dioxo-1,3-dihydrobenzo[*de*]isoquinolin-2-ide.. The product was purified by running on a bed of silica with 5% ethyl acetate, hexane to obtain a yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 7.3 Hz, 2 H), 8.29 (d, *J* = 8.3 Hz, 2 H), 8.02 (d, *J* = 8.7 Hz, 1 H), 7.93 (d, *J* = 7.6 Hz, 1 H), 7.89 (d, *J* = 7.6 Hz, 1 H), 7.85 (s, 1 H), 7.81 (t, *J* = 7.8 Hz, 2 H), 7.51-7.57 (m, 2 H), 7.41 (dd, *J* = 8.6, 2.0 Hz, 1 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.8, 134.6, 133.9, 133.4, 133.1, 132.0, 131.9, 129.5, 128.8, 128.5, 128.1, 128.0, 127.3, 126.9, 126.6, 126.4, 123.1 ppm.

4-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)benzonitrile (5f): Title compound was prepared according to general method described above using 4-bromo benzonitrile and potassium 1,3-dioxo-1,3-dihydrobenzo[*de*]isoquinolin-2-ide. The product was purified by running on a bed of silica with 5% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, *J* = 7.3 Hz, 1 H), 8.31 (d, *J* = 8.4 Hz, 1 H), 7.84 (t, *J* = 7.8 Hz 2 H), 7.81 (d, *J* = 8.0 Hz, 1 H), 7.47 (d, *J* = 8.3 Hz, 1 H).

Preparation of 1-phenylpyrrolidine-2,5-dione (7a): To a DMF suspension of Potassium Succinimide (164 mg, 1.20 mmol) was added Bromo Benzene (157 mg, 1.00 mmol) and $CuSO_4.5H_2O$ (250 mg, 1.00 mmol). The reaction mixture was heated at 150°C for 5 h and monitored the completion reaction by TLC. The reaction mixture initially blue in color changes to green, then in to brown red, leaving residual copper. After the reaction is complete the excess solvent was removed under vacuum and the residue was diluted with 50 ml of dichloromethane, washed with 30 ml 10% HCl, followed by 30ml of water. The organic layer was then dried with anhydrous Sodium sulphate and evaporated the solvent under vacuum, to obtain the product. It

was the run over a bed of silica, using Dichloromethane as solvent to obtain pure product (161mg, 92%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (t, *J* = 7.5 Hz, 2 H), 7.41 (d, *J* = 7.5 Hz, 1 H), 7.28 (d, *J* = 7.8 Hz, 2 H), 2.90 (s, 4 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.6, 132.2, 129.4, 128.9, 126.7, 28.6 ppm.

1-*p*-tolylpyrrolidine-2,5-dione (7b): Title compound was prepared according to general method described above using *p*-bromo toluene and potassium succinimide. The product was purified by running on a bed of silica with 25% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.1 Hz, 1 H), 7.14 (d, *J* = 7.8 Hz, 1 H), 2.84 (s, 4 H), 2.37 (s, 3 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.7, 139.0, 130.1, 129.5, 129.4, 128.9, 126.7, 126.5, 28.6, 21.4 ppm.

1-*m*-tolylpyrrolidine-2,5-dione (7c): Title compound was prepared according to general method described above using *m*-bromo toluene and potassium succinimide. The product was purified by running on a bed of silica with 25% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (t, *J* = 7.6 Hz, 1 H), 7.21 (d, *J* = 7.6 Hz, 1 H), 7.07 (s, 1 H), 7.05 (s, 1 H), 2.88 (s, 5 H), 2.39 (s, 3 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.5, 139.5, 132.0, 129.8, 129.2, 127.3, 123.8, 28.7, 21.6 ppm.

1-(4-chlorophenyl)pyrrolidine-2,5-dione (7d): Title compound was prepared according to general method described above using 1-bromo 4-chloro benzene and potassium succinimide. The product was purified by running on a bed of silica with 25% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45(d, *J* = 8.6Hz, 2 H), 7.25 (d, *J* = 8.6 Hz, 2 H), 2.90 (s, 4 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.1, 134.6, 130.6, 129.6, 127.9, 115.6, 28.6 ppm.

1-(naphthalen-2-yl)pyrrolidine-2,5-dione (7e): Title compound was prepared according to general method described above using 2-bromo naphthalene and potassium succinimide. The product was purified by running on a bed of silica with 25% ethyl acetate, hexane to obtain a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.7 Hz, 1H), 7.89 - 7.84 (m, 2 H), 7.80 (s, 1 H), 7.53 (dt, *J* = 5.4, 3.2 Hz, 2 H), 7.38 (dd, *J* = 8.7, 2.0 Hz, 1 H), 2.95 (s, 5 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.7, 133.4, 133.1, 129.6, 129.4, 128.5, 128.0, 127.2, 126.9, 125.88, 124.1, 28.7 ppm.

4-(2,5-dioxopyrrolidin-1-yl)benzonitrile (7f): Title compound was prepared according to general method described above using 4-bromo benzonitrile and potassium succinimide. The product was purified by running on a bed of silica with 25% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.7 Hz, 1 H), 7.47 (d, *J* = 8.7 Hz, 1 H), 2.89 (s, 2 H) ppm: ^{13}C NMR (400MHz, $CDCl_3$) δ 175.6, 136.1, 133.4, 133.1, 127.4, 127.1, 118.3, 112.2, 28.6 ppm.

1-(4-nitrophenyl)pyrrolidine-2,5-dione (7g): Title compound was prepared according to general method described above using 1-bromo 4-nitro benzene and potassium succinimide. The product was purified by running on a bed of silica with 25% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, *J* = 1.2 Hz, 2 H), 7.60 (d, *J* = 8.0 Hz, 2 H), 2.96 (s, 4 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.4, 147.2, 137.6, 127.1, 124.6, 28.6 ppm.

1-(4-acetylphenyl)pyrrolidine-2,5-dione (7h): Title compound was prepared according to general method described above using 4- bromo acetophenone and potassium succinimide. The product was purified by running on a bed of silica with 50% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.5 Hz, 2 H), 7.46 (d, *J* = 8.5 Hz, 2 H),

2.93 (s, 4 H), 2.62 (s, 3 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 197.2, 176.0, 136.8, 136.2, 129.3, 126.6, 28.7, 26.9 ppm.

Preparation of 5,5-dimethyl-3-phenylimidazolidine-2,4-dione(9a): To a DMF suspension of Potassium salt of 5,5- dimethyl Hydantoin (166 mg, 1.00 mmol) was added Bromo Benzene (157 mg, 1.00 mmol) and $CuSO_4 \bullet 5H_2O$ (250 mg, 1.00 mmol). The reaction mixture was heated at 150°C for 4 h and monitored the completion reaction by TLC. The reaction mixture initially blue in color changes to green, then in to brown red, leaving residual copper. After the reaction is complete the reaction mixture was cooled to room temperature and poured on to ice. The precipitate obtained was washed with 10% HCl Solution, dried and run over a bed of silica with Dichloromethane to obtain pure product (192mg, 94%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (t, *J* = 7.3 Hz, 2 H), 7.35 (d, *J* = 8.3 Hz, 2 H), 7.30 (d, *J* = 7.2 Hz, 1 H), 6.80 (s, 1 H), 1.44 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.6, 156.0, 131.8, 129.3, 128.4, 126.4, 58.9, 25.4 ppm.

5,5-dimethyl-3-*p*-tolylimidazolidine-2,4-dione (9b): Title compound was prepared according to general method described above using *p*-bromo toluene and potassium 4,4-dimethyl-2,5 dioxoimidazolidin-1-ide. The product was purified by running on a bed of silica with 50% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (s, 4 H), 7.04 (s, 1 H), 2.38 (s, 3 H), 1.49 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.8, 156.3, 138.5, 130.0, 129.2, 126.4, 58.9, 25.3, 21.4 ppm.

5,5-dimethyl-3-*m*-tolylimidazolidine-2,4-dione (9c): Title compound was prepared according to general method described above using *m*-bromo toluene and potassium 4,4-dimethyl-2,5 dioxoimidazolidin-1-ide. The product was purified by running on a bed of silica with 50% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (t, *J* = 7.6 Hz, 1

H), 7.20 (s, 2 H), 7.18 (s, 1 H), 2.39 (s, 3 H), 1.48 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.8, 156.2, 139.3, 131.7, 129.4, 129.2, 127.2, 123.7, 58.9, 25.3, 21.6 ppm.

3-(4-chlorophenyl)-5,5-dimethylimidazolidine-2,4-dione (9d): Title compound was prepared according to general method described above using 1-bromo 4-chloro benzene and potassium 4,4-dimethyl-2,5 dioxoimidazolidin-1-ide. The product was purified by running on a bed of silica with 50% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 7.3 Hz, 2 H), 7.38 (d, J = 8.9 Hz, 2 H), 7.21 (s, 1 H), 1.48 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.4, 155.7, 134.1, 130.4, 129.5, 127.6, 59.0, 25.3 ppm.

5,5-dimethyl-3-(naphthalen-2-yl)imidazolidine-2,4-dione (9e): Title compound was prepared according to general method described above using 2-bromo naphthalene and potassium 4,4-dimethyl-2,5 dioxoimidazolidin-1-ide. The product was purified by running on a bed of silica with 50% ethyl acetate, hexane to obtain a pale yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 9.3 Hz, 2 H), 7.91-7.85 (m, 2 H), 7.53 (dd, J = 6.1, 3.1 Hz, 3 H), 6.99 (s, 1 H), 1.53 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 176.7, 156.1, 133.4, 132.9, 129.2, 128.4, 128.0, 127.0, 126.9, 125.5, 124.1, 59.0, 25.4 ppm.

4-(4,4-dimethyl-2,5-dioxoimidazolidin-1-yl)benzonitrile (9f): Title compound was prepared according to general method described above using 4-bromo benzonitrile and potassium 4,4-dimethyl-2,5 dioxoimidazolidin-1-ide. The product was purified by running on a bed of silica with 50% ethyl acetate, hexane to obtain a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.0, 2 H), 7.67 (d, J = 8.0, 2 H), 6.72 (s, 1 H), 1.54 (s, 6 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.8, 154.7, 136.1, 133.1, 126.2, 118.4, 111.5, 59.0, 29.9, 25.5 ppm.

5,5-dimethyl-3-(4-nitrophenyl)imidazolidine-2,4-dione (9g): Title compound was prepared according to general method described above using 1-bromo 4-nitro benzene and potassium 4,4-dimethyl-2,5 dioxoimidazolidin-1-ide. The product was purified by running on a bed of silica with 50% ethyl acetate, hexane to obtain a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (dd, *J* = 8.0, 4.0, 2 H), 7.73 (d, *J* = 8.0, 2 H), 7.08 (s, 1 H) ppm: ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.8, 154.8, 146.6, 137.8, 126.1, 124.5, 59.0, 25.4 ppm.

Preparation of 2-phenyl-1*H*-benzo[*f*]isoindole-1,3(2*H*)-dione (11a): To a DMF suspension of Potassium salt of 2,3 Naphthalimide (282 mg, 1.2 mmol) was added Bromo Benzene (157 mg, 1.0 mmol) and $CuSO_4.5H_2O$ (250 mg, 1.0 mmol). The reaction mixture was heated at 150^{O}c for 5 hours and monitored the completion reaction by TLC. The reaction mixture initially blue in color changes to green, then in to brown red, leaving residual cuprous oxide (Cu_2O). After completion of the reaction, the reaction mixture was cooled to room temperature and poured on to ice. The precipitate obtained was washed with 10% HCl Solution, dried and run over a bed of silica to obtain pure product (230mg, 86%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (s, 2 H), 8.10 (dd, *J* = 6.1, 3.3 Hz, 2 H), 7.73 (dd, *J* = 6.1, 3.3 Hz, 2 H), 7.57 - 7.48 (m, 3 H), 7.43 (t, *J* = 7.0 Hz, 2 H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.3, 135.9, 132.1, 130.6, 129.6, 129.4, 128.4, 127.7, 126.9, 125.5 ppm.

2-*p*-tolyl-1*H*-benzo[*f*]isoindole-1,3(2*H*)-dione (11b): Title compound was prepared according to general method described above using *p*-bromo toluene and potassium 1,3-dioxo-1,3-dihydrobenzo[*f*]isoindol-2-ide. The product was purified by running on a bed of silica with 10% ethyl acetate, hexane to obtain a pale yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 2 H), 8.09 (dd, *J* = 6.1, 3.3 Hz, 2 H), 7.72 (dd, *J* = 6.2, 3.3 Hz, 2 H), 7.37 (d, *J* = 8.3 Hz, 2 H), 7.33

(d, J = 8.3 Hz, 2 H), 2.42 (s, 3 H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ167.4, 138.5, 135.9, 130.6, 130.0, 129.5, 129.4, 127.8, 126.7, 125.4 ppm.

2-(naphthalen-2-yl)-1*H*-benzo[*f*]isoindole-1,3(2*H*)-dione (11c): Title compound was prepared according to general method described above using 2-bromo naphthalene and potassium 1,3-dioxo-1,3-dihydrobenzo[*f*]isoindol-2-ide. The product was purified by running on a bed of silica with 10% ethyl acetate, hexane to obtain a yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 2 H), 8.12 (dd, J = 6.0, 3.2 Hz, 2 H), 8.00 (d, J = 9.1 Hz, 2 H), 7.91 (dd, J = 8.5, 4.2 Hz, 2 H), 7.75 (dd, J = 6.0, 3.2 Hz, 2 H), 7.61 (d, J = 8.7 Hz, 1 H), 7.55 (dd, J = 5.7, 3.6 Hz, 2 H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ 167.4, 136.0, 133.5, 132.9, 130.6, 129.6, 129.6, 129.2, 128.5, 128.0, 127.7, 127.0, 126.8, 125.9, 125.6, 124.4 ppm.

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Chapter-6: $CuSO_4 \bullet 5H_2O$ Mediated Coupling of Aryl Halides With N-Formyl Amines

6.1 Introduction:

Transition metal catalyzed C-N bond formations are one of the revolutionized reactions in the modern era of organic synthesis [(1)], especially arylation of amines plays an important role in the modern era of organic synthesis because of its use in synthesizing including drugs, materials, natural products agro chemicals and optical devices [(2).]

Copper was the first metal used by Ullmann and Goldberg for the construction of C-N bond [(3)]. However the use of high temperatures and low percentage yields with long reaction timings and finally lack substrate tolerability [(4)] force us to develop the new methods for the cross coupling reactions.

In recent literature we have some references where they are producing some good results in the arylation of amines with Cu catalysis by using external ligands such as diols [(5)], trios [(6)], rac-binols [(7)], diamines [(8)], imines [(9)], amino phosphates [(10)], amino acids [(11)], amino alcohols [(12)], salicyl amides [(13)], phosphoramidites [(14)], oxime- phosphine oxides [(15)], pyridine N- oxide [(16)], beta diketones [(17)], beta ketoesters [(18)], and diazaphospholanes [(19)]. But including the difficulties in the synthesis of these ligands and of purification problems we are in need of developing a good catalytic system for aryl amination reactions.

6.2 Results and Discussion:

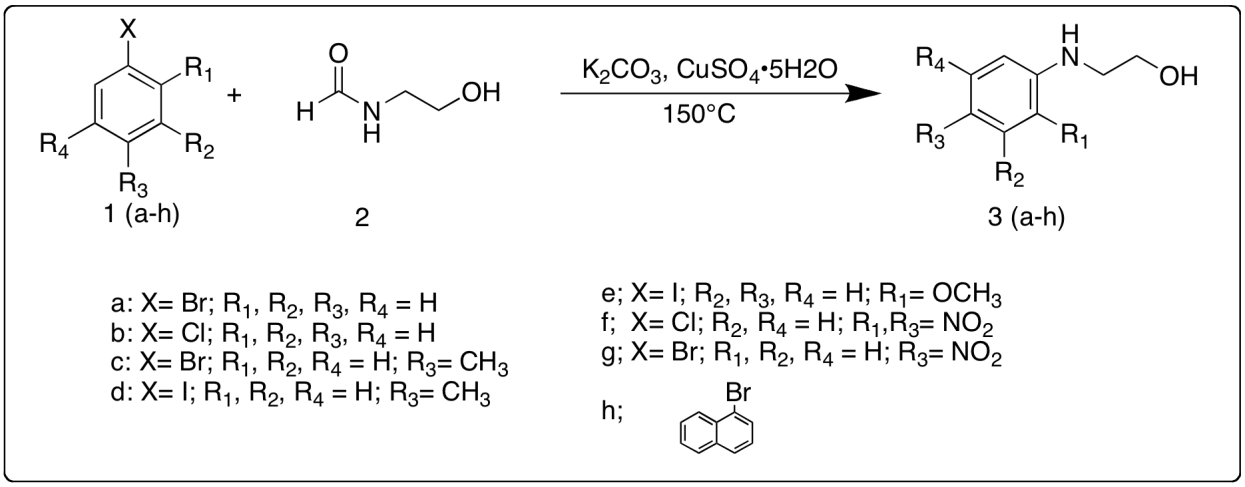
I started working on N-arylation of amino alcohols because of their importance in the medicinal chemistry and in organo metallic chemistry [20], in this contest I found a method in the literature developed by Buchwald group by using CuI but this method limited to few substrates only [21].

We also have some references where we can use amides as N-nucleophiles for cross coupling reactions, and they achieved success with Pd [22]. However, high cost of Pd and use of external ligands it is very hard to apply these reactions in the industrial scale, it is important to mention that removing Pd residues from the reaction media requires special methods, which increase its cost furthermore.

6.2.1 Aryl halides coupling with N-formyl-2-aminoethanol

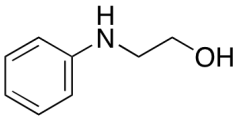
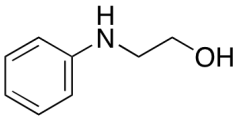
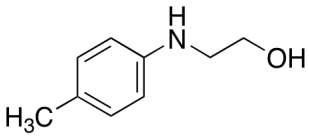
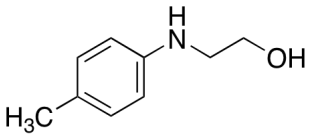
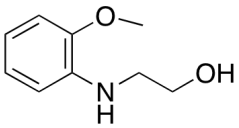
I started coupling of simple N-formyl ethanolamine with aromatic halides in the presence of $CuSO_4 \bullet 5H_2O$ catalysis (Scheme 6.1). The coupling reaction required relatively short reaction time and ends with moderate to excellent yields. It is important to mention that, the reaction doesn't require any additional solvent, because N-formyl amines act as solvent as well as substrates.

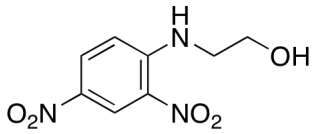
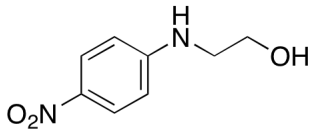
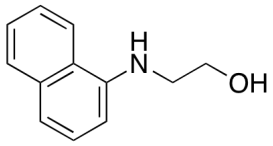
The reaction has a broad scope of substrates including simple bromo benzene, to electron rich aromatic halides such as 2-iodo anisole, 4- methyl benzene and strong electron deficient halides like 4-bromo nitrobenzene, 1-chloro-2, 4-dinitro benzene. The most important thing we are selectively getting N-arylation over o-arylation.



Scheme 6.1 Aryl halides coupling with N-formyl-2-aminoethanol

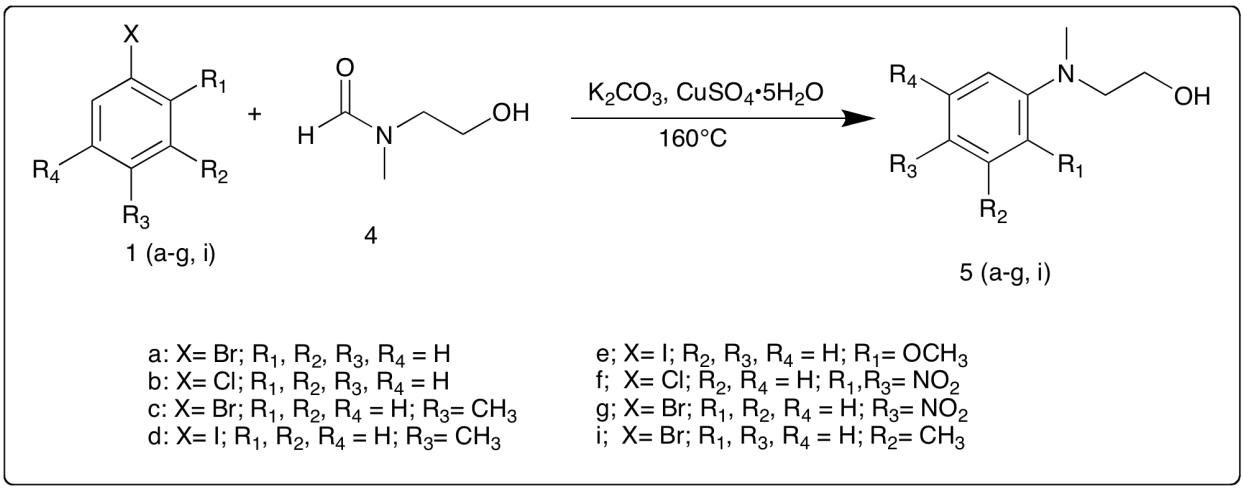
Table 6.1 Prepared N-aryl-2-aminoethanol derivatives

Entry #	Product Structure	Reaction Time (h)	% Yield
3a		4	80
3b		4	78
3c		4	84
3d		4	89
3e		4	95

3f		4	88
3g		4	95
3h		4	90

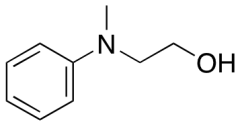
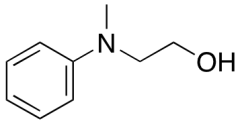
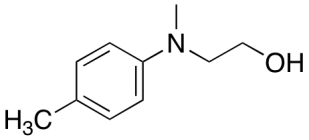
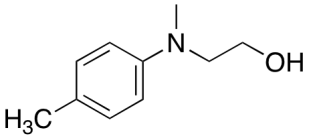
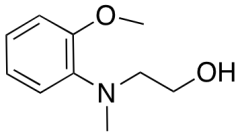
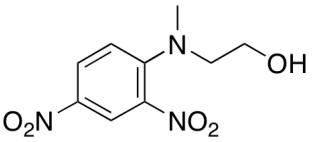
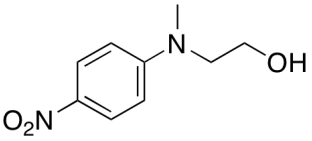
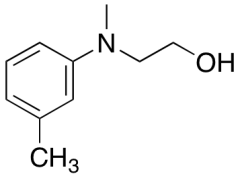
6.2.2 Aryl halides coupling with N-formyl-2-(methylamino)ethanol

After getting decent results with N-formyl ethanolamine, I would like expand the scope of methodology to the 2^{o} amines such as N,N-methyl formyl ethanolamine (Scheme 6.2), and ended up with similar results except in this case electron deficient aromatic halides requires less reaction timings then electron rich aromatic halides.



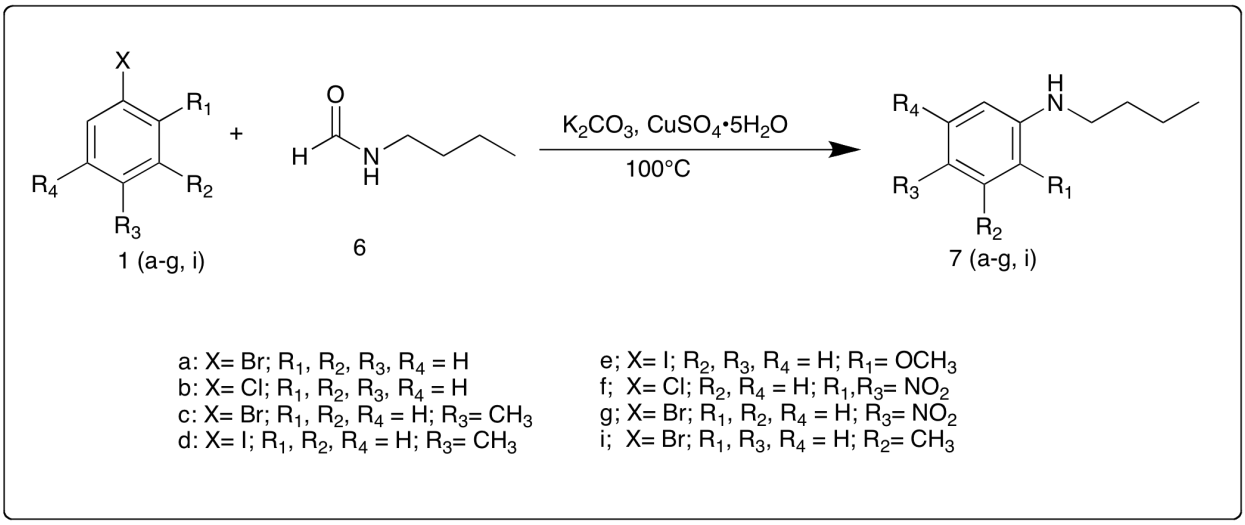
Scheme 6.2 Aryl halides coupling with N-formyl-2-(methylamino)ethanol

Table 6.2 Prepared N-aryl-2-(methylamino)ethanol derivatives

Entry#	Product	Reaction Time (Hrs)	% Yield
1		4	87
2		4	82
3		6	80
4		4.5	85
5		2.5	91
6		1.5	82
7		3	93
8		2.5	91

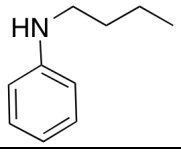
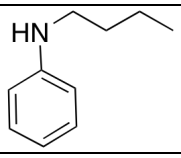
6.2.3 Aryl halides coupling with N-formylbutylamine

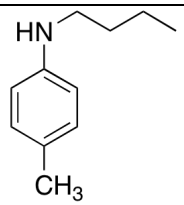
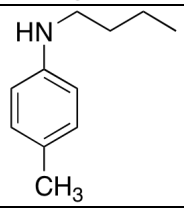
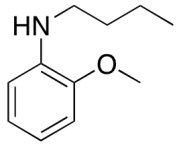
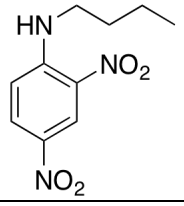
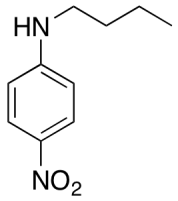
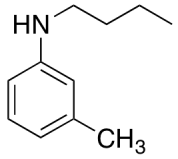
To expand the scope the reaction furthermore, I moved onto the long chain amines such as N-formyl butyl amine (Scheme 6.3) and N-formyl nonylamine, and found that the reaction timings are very high for N-formyl butyl amine and in the case of N-formyl nonylamine only 1-chloro-2,4-dinitrobenzene is reacting (Scheme 6.4). We thought that the low boiling points of butyl amine and nonyl amine are the reason for long reaction times.



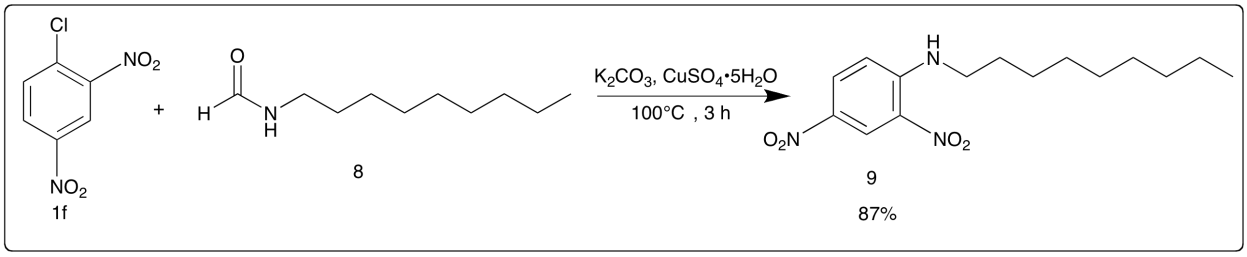
Scheme 6.3 Aryl halides coupling with N-formylbutylamine

Table 6.3 Prepared N-arylbutylamine derivatives

Entry #	Product Structure	Reaction Time (h)	% Yield
7a		24	65
7b		24	60

7c		24	73
7d		24	84
7e		24	87
7f		12	95
7g		24	85
7i		24	76

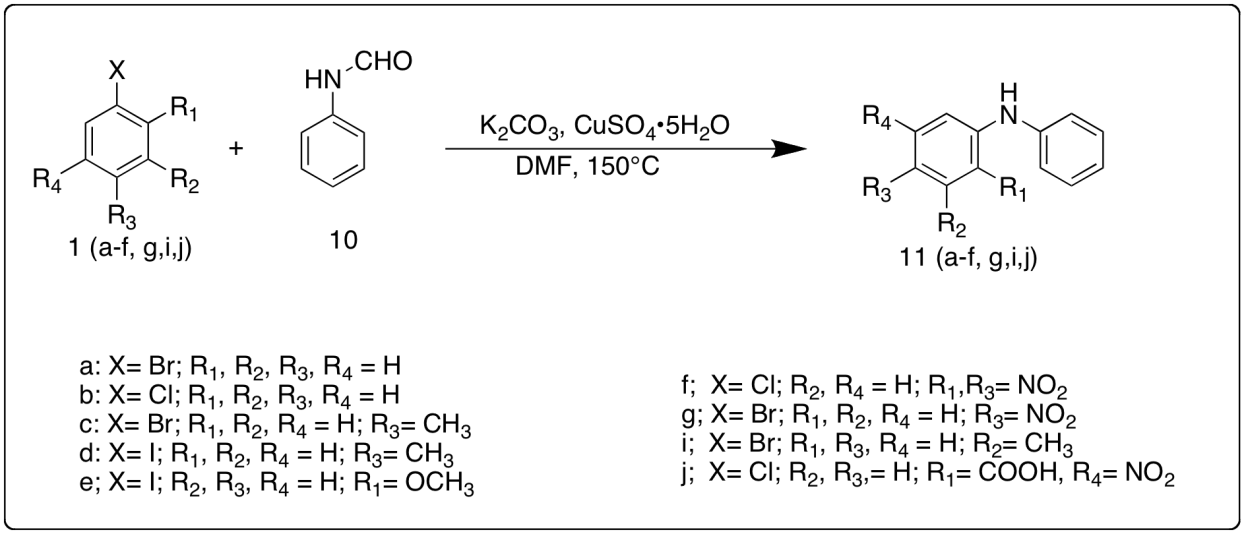
6.2.4 Aryl halides coupling with N-formyl nonylamine



Scheme 6.4 Aryl halides coupling with N-formylnonylamine

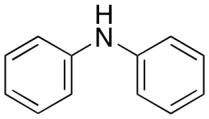
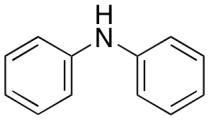
6.2.5 Aryl halides coupling with N-formyl aniline

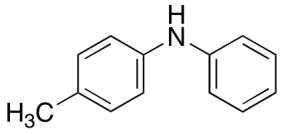
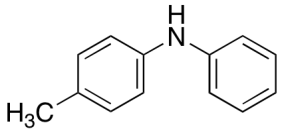
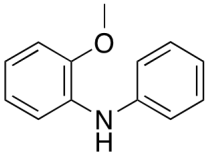
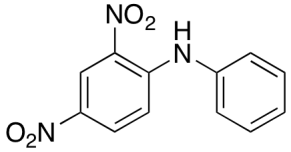
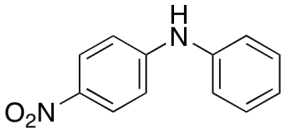
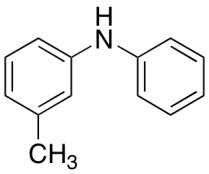
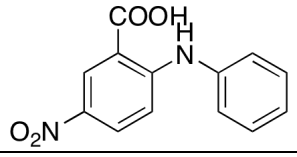
So far we have seen the coupling reactions only with aliphatic amines such as ethanolamine, methyl ethanolamine, butyl amine and nonyl amine. I also screened the reaction on aromatic amines such as N-formyl aniline (Scheme 6.5). However, the aryl halides coupling with N-formy anilines required additional solvents like DMF, because of the reported aliphatic amines are liquids where as N-formyl aniline is a solid.



Scheme 6.5 Aryl halides coupling with N-formyl aniline

Table 6.4 Prepared N-aryl aniline derivatives

Entry #	Product Structure	Reaction Time (h)	% Yield
11a		18	91
11b		18	89

11c		24	76
11d		18	83
11e		12	91
11f		12	89
11g		12	83
11i		24	73
11j		12	86

6.3 Conclusions:

In conclusion we developed a very good method for N-arylation of amines by using N-formyl amines as nitrogen nucleophiles. The N-arylation is achieved by coupling of aryl halides with N-formyl amines by using $CuSO_4 \bullet 5H_2O$. The methodology has the selectivity of N-arylation over O-arylation while coupling the ethanolamines with aryl halides. It also had the broad applicability to various amines such as primary (2-ethanolamine), secondary (2-(methylamino)ethanol)) and aromatic amines (aniline).

6.4 Experimental Section:

General procedure for N-formylation of amines: Synthesis of N-formyl-2-ethanolamine (2):

Ethanolamine (30.5 g, 0.500 mol) was dissolved in (53.6 g, 0.500 mol) of trimethyl orthoformate and to this solution (2000 mg, 10.50 mmol) *p*-toluenesulfonic acid and few drops of water is added, the whole reaction mixture is stirred in a microwave at a power of 200 about over night. Bring down the temperature to room temperature and the formed N-formy-2-ethanolamine was used directly for the coupling reactions without further purification.

General procedure for coupling of aryl halides with N-formyl-2-ethanolamine:

Synthesis of 2-(*p*-tolylamino)ethanol (3c):

p-bromotoluene (342 mg, 2.00 mmol), (306 mg, 2.20 mmol) of potassium carbonate and (500 mg, 2.00 mmol) of $CuSO_4 \bullet 5H_2O$ were dissolved in 12 ml of N-formyl-2-ethanolamine and the whole reaction mixture was stirred at 150°C about 4 h and monitored the reaction progress by

TLC. After completion of the reaction cool it down to the room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 254 mg of 2-(*p*-tolylamino)ethanol (**3c**) with 84% yield.

General procedure for aryl halides coupling with N-formyl-2-(methylamino)ethanol:

Synthesis of 2-(methyl(*p*-tolyl)amino)ethanol (5c):

p-bromotoluene (342 mg, 2.00 mmol) , (306 mg, 2.20 mmol) of potassium carbonate and (500 mg, 2.00 mmol) of $CuSO_4 \bullet 5H_2O$ were dissolved in 12 ml of N-formyl-2-(methylamino)ethanol and the whole reaction mixture was stirred at 160°C about 6 h and monitored the reaction progress by TLC. After completion of the reaction cool it down to the room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 264 mg of 2-(methyl(*p*-tolyl)amino)ethanol (**5c**) with 80% yield.

General procedure for aryl halides coupling with N-formylbutylamine:

Synthesis of N-butyl-4-methylaniline (7c):

p-bromotoluene (342 mg, 2.00 mmol) , (306 mg, 2.20 mmol) of potassium carbonate and (500 mg, 2.00 mmol) of $CuSO_4 \bullet 5H_2O$ were dissolved in 12 ml of N-formyl-2-(methylamino)ethanol and the whole reaction mixture was stirred at 100°C about 24 h and monitored the reaction progress by TLC. After completion of the reaction cool it down to the room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted

the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 228 mg of N-butyl-4-methylaniline (**7c**) with 70% yield.

General procedure for aryl halides coupling with N-formylnonylamine:

Synthesis of 2,4-dinitro-N-nonylamine (9):

1-chloro-2,4-dinitrobenzene (505 mg, 2.50 mmol), (382 mg, 2.70 mmol) of potassium carbonate and (613 mg, 2.50 mmol) of $CuSO_4 \bullet 5H_2O$ were dissolved in 12 ml of N-formyl nonylamine and the whole reaction mixture was brought to reflux about 3 h, after that cool it down to the room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 673 mg of 2,4-dinitro-N-nonylamine with 87% yield.

General procedure for aryl halides coupling with N-formylaniline:

Synthesis of 4-methyl-N-phenylaniline (11c):

N-formyl aniline (660 mg, 2.20 mmol) was dissolved in 10 ml of DMF, to this stirring solution (342 mg, 2.00 mmol) of *p*-bromotoluene, (306 mg, 2.20 mmol) of potassium carbonate and (500 mg, 2.00 mmol) of $CuSO_4 \bullet 5H_2O$ were added and the whole reaction mixture was stirred at 150°C about 24 h and monitored the reaction progress by TLC, after completion of reaction cool it down to the room temperature and washed with water thoroughly and one time wash with 1% HCl solution, and finally extracted the organic layer with DCM. Combined layers were dried under sodium sulfate and a flash column required getting 278 mg of 4-methyl-N-phenylaniline (**11c**) with 76% yield.

6.5 Spectral Section:

2-(phenylamino)ethanol: (3a & 3b): ^{1}H NMR ($CDCl_3$): δ 7.20 (t, *J* = 7.2 Hz, 2 H), 6.76 (t, *J* = 7.2 Hz, 1 H), 6.69 (d, *J* = 8.0 Hz, 2 H), 3.84 (t, *J* = 4.4 Hz, 2 H), 3.32 (t, *J* = 5.2 Hz, 2 H), 2.72 (b, 2 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.1, 129.6, 118.5, 113.7, 61.4, 46.6 ppm.

2-(*p*-tolylamino)ethanol(3c & 3d): ^{1}H NMR ($CDCl_3$): δ 7.01 (d, *J* = 8.0 Hz, 2 H), 6.59 (d, *J* = 8.0 Hz, 2 H), 3.79 (t, *J* = 4.8 Hz, 2 H), 3.25 (t, *J* = 5.2 Hz, 2 H), 3.08 (b, 2 H), 2.26 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 146.1, 130.1, 127.5, 113.8, 61.4, 46.8, 20.2 ppm.

2-(2-methoxyphenylamino)ethanol(3e): ^{1}H NMR ($CDCl_3$): δ 6.88 (t, *J* = 7.6 Hz, 1 H), 6.79 (d, *J* = 7.6 Hz, 1 H), 6.72 (d, *J* = 8.0 Hz, 1 H), 6.68 (t, *J* = 6.0 Hz, 1 H), 3.84 (t, *J* = 5.6 Hz, 2 H), 3.32 (t, *J* = 4.8 Hz, 2 H) ppm; ^{13}C NMR ($CDCl_3$): δ 147.4, 138.1, 121.5, 117.4, 110.6, 109.8, 61.5, 55.7, 46.2 ppm.

2-(2,4-dinitrophenylamino)ethanol(3f): ^{1}H NMR ($CDCl_3$) : δ 9.14 (s, 1 H), 8.82 (b, 1 H), 8.28 (d, *J* = 9.6 Hz, 1 H), 6.98 (d, *J* = 9.6 Hz, 1 H), 4.02 (t, *J* = 5.6 Hz, 2 H), 3.6 (q, *J* = 10.8, 5.6 Hz, 2 H), 1.64 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.8, 130.6, 124.6, 114.3, 60.7, 45.4 ppm.

2-(4-nitrophenylamino)ethanol(3g): ^{1}H NMR ($CDCl_3$): δ 8.09 (d, *J* = 9.2 Hz, 2 H), 6.57 (d, *J* = 9.2 Hz, 2 H), 3.91 (t, *J* = 5.2 Hz, 2 H), 3.40 (t, *J* =4.8 Hz, 2 H), 1.63 (b, 2 H) ppm; ^{13}C NMR ($CDCl_3$): δ 126.7, 111.6, 61.1, 45.4 ppm.

2-(naphthalen-1-ylamino)ethanol(3h): ^{1}H NMR ($CDCl_3$): δ 7.84 (q, *J* = 14.0, 8.0 Hz, 2 H), 7.46 (p, *J* = 5.6 Hz, 2 H), 7.37 (t, *J* = 7.6 Hz, 1 H), 7.30 (d, *J* = 8.4 Hz, 1 H), 6.62 (d, *J* = 8.0 Hz, 1 H), 3.90 (t, *J* = 4.8 Hz, 2 H), 3.38 (t, *J* = 4.8 Hz, 2 H) ppm; ^{13}C NMR ($CDCl_3$): δ 143.6, 134.6, 128.9, 126.8, 126.1, 125.1, 124.0, 120.3, 118.2, 105.1, 61.2, 46.4 ppm.

2-(methyl(phenyl)amino)ethanol (5a & 5b): ^{1}H NMR ($CDCl_3$): 7.26 (t, *J* = 7.6 Hz, 2 H), 6.82 (d, *J* = 8.4 Hz, 2 H), 6.77 (t, *J* = 7.2 Hz, 1 H), 3.81 (t, *J* = 5.6 Hz, 2 H), 3.47 (t, *J* = 5.6 Hz, 2 H), 2.97 (s, 3 H), 2.06 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 150.3, 129.5, 117.5, 113.4, 60.3, 55.7, 39.1 ppm.

2-(methyl(*p*-tolyl)amino)ethanol (5c & 5d): ^{1}H NMR ($CDCl_3$); δ 7.07 (d, *J* = 8.0 Hz, 2 H), 6.76 (d, *J* = 8.4 Hz, 2 H), 3.79 (t, *J* = 5.6 Hz, 2 H), 3.41 (t, *J* = 5.6 Hz, 2 H), 2.92 (s, 3 H), 2.28 (s, 3 H), 2.11 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.5, 130.0, 127.2, 114.1, 60.2, 56.3, 39.2, 20.5 ppm.

2-((2-methoxyphenyl)(methyl)amino)ethanol (5e): ^{1}H NMR ($CDCl_3$): δ 7.04 (t, *J* = 7.6 Hz, 1 H), 7.00 (d, *J* = 8.0 Hz, 1 H), 6.91 (t, *J* = 7.6 Hz, 1 H), 6.86 (d, *J* = 7.6 Hz, 1 H), 3.84 (s, 3 H), 3.71 (t, *J* = 5.2 Hz, 2 H), 3.24 (b, 1 H), 3.11 (t, *J* = 5.2 Hz, 2 H), 2.78 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 152.9, 142.1, 123.6, 121.2, 120.4, 111.4, 59.7, 58.4, 55.5, 40.1 ppm.

2-((2,4-dinitrophenyl)(methyl)amino)ethanol (5f): ^{1}H NMR ($CDCl_3$): δ 8.65 (s, 1 H), 8.19 (d, *J* = 9.2 Hz, 1 H), 7.22 (d, *J* = 9.6 Hz, 1 H), 3.91 (t, *J* = 5.2 Hz, 2 H), 3.61 (t , *J* = 5.2 Hz, 2 H), 2.99 (s, 3 H), 1.87 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 149.8, 136.8, 127.8, 124.2, 118.90, 59.5, 56.2, 40.8 ppm.

2-(methyl(4-nitrophenyl)amino)ethanol (5g): ^{1}H NMR ($CDCl_3$): δ 8.00 (d, *J* = 9.6 Hz, 2 H), 6.62 (d, *J* = 9.2 Hz, 2 H), 3.86 (t, *J* = 5.6 Hz, 2 H), 3.62 (t, *J* = 6.0 Hz, 2 H), 3.12 (s, 3 H), 2.2 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 154.1, 137.0, 126.4, 110.7, 60.2, 54.7, 39.7 ppm.

2-(methyl(m-tolyl)amino)ethanol (5i): ^{1}H NMR ($CDCl_3$): δ 7.15 (t, *J* = 7.6 Hz, 1 H), 6.66-6.60 (m, 3 H), 3.81 (t, *J* = 5.6 Hz, 2 H), 3.46 (t, *J* = 5.6 Hz, 2 H), 2.95 (s, 3 H), 2.33 (s, 3 H), 2.0 (b,

1H) ppm; ^{13}C NMR ($CDCl_3$): δ 150.4, 139.2, 129.3, 118.6, 114.3, 110.7, 60.3, 55.8, 39.1, 22.1 ppm.

N-butylaniline (7a & 7b): ^{1}H NMR ($CDCl_3$): δ 7.19 (t, *J* = 8.4 Hz, 2 H), 6.71 (t, *J* = 7.2 Hz, 1 H), 6.62 (d, *J* = 8.4 Hz, 2 H), 3.6 (b, 1 H), 3.13 (t, *J* = 7.2 Hz, 2 H), 1.63 (q, *J* = 7.2 Hz, 2 H), 1.46 (Sextet, *J*= 15.2, 7.6 Hz, 2 H), 0.98 (t, *J* = 7.6 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.8, 129.5, 117.3, 112.9, 43.9, 31.9, 20.6, 14.2 ppm.

N-butyl-4-methylaniline (7c & 7d): ^{1}H NMR ($CDCl_3$): δ 7.08 (d, *J* = 7.6 Hz, 2 H), 6.63 (d, *J* = 8.4 Hz, 2 H), 3.46 (b, 1 H), 3.17 (t, *J* = 6.8 Hz, 2 H), 2.34 (s, 3 H), 1.68 (p, *J* = 6.8 Hz, 2 H), 1.53 (p, *J* = 7.6 Hz, 2 H), 1.06 (t, *J* = 7.2 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 146.7, 130.0, 126.6, 113.2, 44.4, 32.0, 20.6, 14.3 ppm.

N-butyl-2-methoxyaniline (7e): ^{1}H NMR ($CDCl_3$): δ 6.89 (t, *J* = 7.6 Hz, 1 H), 6.78 (d, *J* = 8.0 Hz, 1 H), 6.68 (d, *J* = 7.6 Hz, 1 H), 6.64 (t, *J* = 7.6 Hz, 1 H), 4.20 (b), 3.86 (s, 3 H), 3.14 (t, *J* = 7.2 Hz, 2 H), 1.66 (p, *J* = 7.6 Hz, 2 H), 1.47 (Sextet, *J* = 14.8 Hz, 7.6, 2 H), 0.98 (t, *J* = 7.2 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 147.0, 138.8, 121.6, 116.3, 110.0, 109.6, 55.6, 43.6, 31.9, 20.6, 14.2 ppm.

N-butyl-2,4-dinitroaniline (7f): ^{1}H NMR ($CDCl_3$) ; δ 9.03 (s, 1 H), 8.53 (b), 8.21 (d, *J* = 9.6 Hz, 1 H), 6.92 (d, *J* = 9.6 Hz, 1 H), 3.41 (q, *J* = 12.4 Hz, 7.2, 2 H), 1.75 (p, *J* = 7.2 Hz, 2 H), 1.48 (Sextet, *J* = 15.2 Hz, 8.0, 2 H), 0.98 (t, *J* = 7.2 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.7, 136.0, 130.5, 130.3, 124.4, 114.3, 43.6, 30.9, 20.3, 13.9 ppm.

N-butyl-4-nitroaniline (7g): ^{1}H NMR ($CDCl_3$); δ 8.03 (d, *J* = 9.2 Hz, 2 H), 6.49 (d, *J* = 9.2 Hz, 2 H), 4.78 (b, 1 H), 3.17 (q, *J* = 12.8 Hz, 7.2, 2 H), 1.61 (p, *J* = 7.6 Hz, 2 H), 1.27 (Sextet, *J* =

15.2, 7.2 Hz, 2 H), 0.93 (t, *J* = 7.6 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 154.0, 137.6, 126.7, 111.1, 43.3, 31.3, 20.4, 14.0 ppm.

N-butyl-3-methylaniline (7i): ^{1}H NMR ($CDCl_3$): δ 7.13 (t, J = 7.6, 1 H), 6.58 (d, j = 8.0, 1 H), 6.49 (d, j = 7.2, 2 H), 3.47 (b, 1 H), 3.16 (t, j = 7.2, 2 H), 2.34 (s, 3 H), 1.65 (p, j = 7.2, 2 H), 1.49 (Sextet, j = 15.2, 7.6, 2 H), 1.02 (t, J = 7.6, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.9, 139.2, 129.4, 118.3, 113.8, 110.2, 44.0, 32.0, 21.9, 20.6, 14.2 ppm.

2,4-dinitro-N-nonylaniline (9): ^{1}H NMR ($CDCl_3$): δ 9.12 (s, 1 H), 8.56 (b, 1 H), 8.26 (d, *J* = 9.6 Hz, 1 H), 6.92 (d, *J* = 9.6 Hz, 1 H), 3.40 (q, *J* = 12.4 Hz, 6.8, 2 H), 1.77 (p, *J* = 7.6 Hz, 2 H), 1.46-1.27 (m, 12 H), 0.87 (t, *J* = 6.8 Hz, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 148.6, 136.1, 130.6, 130.4, 124.6, 114.1, 43.9, 32.0, 29.6, 29.4, 28.9, 27.2, 22.9, 14.3 ppm.

Diphenylamine (11a & 11b): ^{1}H NMR ($CDCl_3$): δ 7.33 (t, *J* = 8.4 Hz, 4 H), 7.13 (d, *J* = 8.4 Hz, 4 H), 7.00 (d, *J* = 8.0 Hz, 2 H), 5.70 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 143.4, 129.7, 121.3, 118.1 ppm.

4-methyl-N-phenylaniline (11c & 11d): ^{1}H NMR ($CDCl_3$): δ 7.35 (t, *J* = 8.8 Hz, 2 H), 7.20 (d, *J* = 8.02 Hz, 2 H), 7.13-7.10 (m, 4 H), 7.01 (t, *J* = 7.2 Hz, 1 H), 5.60 (b, 1 H), 2.43 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 144.30, 140.7, 131.24, 130.2, 129.7, 120.7, 119.3, 117.2, 21.1 ppm.

2-methoxy-N-phenylaniline (11e): ^{1}H NMR ($CDCl_3$): δ 7.43 (t, *J* = 6.8 Hz, 1 H), 7.39 (d, *J* = 7.2 Hz, 2 H), 7.27 (d, *J* = 8.0 Hz, 2 H), 7.06 (t, *J* = 8.0 Hz, 1 H), 7.02-6.99 (m, 3 H), 6.35 (b, 1 H), 3.96 (s, 3 H), ppm; ^{13}C NMR ($CDCl_3$): δ 148.5, 143.0, 133.2, 129.6, 121.4, 121.1, 120.2, 118.8, 114.9, 110.8, 55.8 ppm.

2,4-dinitro-N-phenylaniline(11f): ^{1}H NMR ($CDCl_3$): δ 9.98(b, 1 H), 9.16 (s, 1 H), 8.16 (d, *J* = 6.0 Hz, 1 H), 7.51 (t, *J* = 7.6 Hz, 2 H), 7.39 (t, *J* = 7.2 Hz, 1 H), 7.31 (d, *J* = 7.2 Hz, 2 H), 7.17 (d, *J* = 9.6 Hz, 1 H), 1.60 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 147.4, 137.6, 136.9, 131.3, 130.5, 130.2, 130.0, 125.8, 124.3, 116.3 ppm.

4-nitro-N-phenylaniline (11g): ^{1}H NMR ($CDCl_3$): 8.12 (d, *J* = 9.2 Hz, 2 H), 7.39 (t, *J* = 7.6 Hz, 2 H), 7.21 (d, *J* = 7.6 Hz, 2 H), 7.17 (t, *J* = 6.4 Hz, 1 H), 6.94 (d, *J* = 9.2 Hz, 2 H), 6.34 (b, 1 H) ppm; ^{13}C NMR ($CDCl_3$): δ 150.4, 140.1, 139.7, 130.0, 126.5, 124.9, 122.2, 113.9 ppm.

5-methyl-N-phenylaniline (11i): ^{1}H NMR ($CDCl_3$): δ 7.32 (t, *J* = 7.6 Hz, 2 H), 7.21 (t, *J* = 8.4 Hz, 1 H), 7.11 (d, *J* = 8.4 Hz, 2 H), 6.99 (d, *J* = 7.2 Hz, 1 H), 6.94 (t, *J* = 5.2 Hz, 2 H), 6.81 (d, *J* = 7.2 Hz, 1 H) 5.73 (b, 1 H), 2.36 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$): δ 143.5, 143.3, 139.5, 129.6, 129.5, 122.2, 121.2, 118.8, 118.1, 115.2, 21.8 ppm.

5-nitro-2-(phenylamino)benzoic acid (11j): ^{1}H NMR (dmso): δ 9.75 (s, 1 H), 8.09 (d, *J* = 8.8 Hz, 1 H), 7.79 (s, 1 H), 7.47 (d, *J* = 7.6 Hz, 1 H), 7.43 (t, *J* = 7.6 Hz, 2 H), 7.31 (d, *J* = 8.4 Hz, 2 H), 7.19 (t, *J* = 7.2 Hz, 1 H), 3.6-3.2 (b, 1 H) ppm; ^{13}C NMR (dmso): δ 169.4, 151.5, 148.5, 139.9, 134.3, 130.5, 125.4, 123.4, 117.7, 111.4, 108.2 ppm.

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