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Design and Synthesis of Trisubstituted Pyrimidine Derivatives as Coactivator Binding Inhibitors (CBIs) of Estrogen Receptor Signaling

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Design and Synthesis of Trisubstituted Pyrimidine Derivatives as Coactivator Binding Inhibitors (CBIs) of Estrogen Receptor Signaling

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Thesis Approval

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Dedication

I am deeply grateful and thankful to my husband Alaa Fakhouri; he's always pushing me to succeed and do greatness and never allowing me to give up or doubt myself. For his lovely patience in all my stress and difficult moments, thank you for your endless love and support. This could not have happened without you.

I shall forever remain thankful to my family for the support and help they provided me through my entire life and in particular, I must acknowledge my father, mother and my sweet sister Anmar, without their love and support, I would not have finished this thesis.

I am deeply thankful to my children Raghad and Ameer for their love and patience they surrounded me with.

Declaration:

I certify that this thesis submitted for the degree of Master in Pharmaceutical Science is the result of my own research, except where otherwise acknowledged, and this (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed: …….

Miran M. Jamal Talal Masswadeh

Date: 9-1-2016

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Thanks to Allah, the merciful, the compassionate for granting me strength, the endurance and courage to complete this work .

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I would also like to acknowledge my lab group at the Anticancer Drugs Research Lab, for their help and experimental assistance, working with you has been a real pleasure to me .

Finally, I would like to thank everyone who helped and encouraged me throughout this research.

Abstract:

Breast cancer (BC) is one of the most commonly diagnosed cancers in women. Estrogen signaling and the estrogen receptors (ERα, ERβ) are implicated in breast cancer progression. Majority of breast cancers start out as estrogen dependent as a result of overexpression of ER-regulated genes. Estrogen deprivation therapy using anti-estrogens (AEs) and aromatase inhibitors (AIs) to block ER activity and arrest the estrogen-dependent growth of BC still represents the primary treatment for breast cancer patients. This approach, however, frequently fails and patients develop resistant breast cancer, which is almost untreatable. In this project we focused on synthesizing a potent coactivator binding inhibitors (CBIs) molecules that block ER activity through a different mechanism that may arrest the estrogen-dependent growth of BC and offer a solution to the existing resistance. Co-activator binding inhibitors (CBIs) act by blocking the conformational change needed for DNA binding and gene expression. In this project set of compounds have been designed, synthesized through sequential substitution of the chlorine atoms of 2,4,6-trichloropyrimidine with amines or other nucleophiles. The synthesized compounds were purified using chromatography techniques, and characterized by (^{1}H-NMR, ^{13}C-NMR, FT-IR and MS (ESI)) spectroscopy. Some of these compounds were screened for their inhibitory activity against the acute myeloid leukemia cells (Molm-13) cells. Two forms of Molm-13 had been used to evaluate the role of p53. In one case cells were transfected with empty vector and in the other the cells were tranfected with sh-p53 RNA (sh-p53). The viability of cells was determined using WST-1 assay.

Initial results of the tested compounds demonstrated that MY12 **(35)** and MY3 **(26)** exhibited potent activities against the two forms of Molm-13 cell lines and have a dose dependent effect while the compound MY2 **(25)** showed no significant inhibitory action on the same cell lines.

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List of Abbreviations:

Abbreviation	Full Form
AF	Activation function
AML	Acute Myeloid Leukemia
AR	Androgen receptor
ATR	Ataxia telangiectasia and Rad 3 related kinase
BC	Breast cancer
BOC-pz	Piperazine-1-carboxylic acid *tert*-butyl ester
CARM	Coactivator associated arginine methyltransferase
CBI	Coactivator binding inhibitors
$CDCl_3$	Chloroform-d
CDK	Cyclin dependent kinase
$CHCl_3$	Chloroform
CML	Chronic Myeloid Leukemia
CNS	Central nervous system (CNS)
D_2O	Deuterium oxide
DBD	DNA-binding domain
DCM	Dichloromethane
DHFR	Dihydrofolate reductase
DIPEA	Diisopropylethylamine
DMSO-d_6	Deuterated dimethyl sulfoxide (d6)
DNA	Deoxyribonucleic acid
E2	17 β--estradiol
EGFR	Epidermal growth-factor receptor

EphA2	Ephrin type-A receptor 2
EphB4	Ephrin type-B receptor 4
ER	Estrogen receptor
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
Eth.Ac.	Ethyl acetate
EtOH	Ethanol
FAK	Focal adhesion kinase
FLT3	FMS-like tyrosine kinase-3
FLT3-ITD	FMS-like tyrosine kinase 3 with internal tandem duplication
FTIR	Fourier transform infrared spectroscopy
GC	Glucocorticoid
GIST	Gastrointestinal stromal tumours
GSK3b	Glycogen synthase kinase-3b gene
HAT	Histone acetyltransferase
HCl	Hydrochloric acid
HDAC	Histone deacetylase
HER-2	Human epidermal growth factor receptor-2
HER-3	Human epidermal growth factor receptor 3
HER-4	Receptor tyrosine-protein kinase erbB-4
HSP90	Heat shock protein 90
HXMS	Hydrogen exchange mass spectrometry
JAK	Janus kinase
K_2CO_3	Potassium carbonate
KB	Potassium bromide

Kdr	Kinase insert domain receptor
LBD	Ligand-binding domain
LBP	Ligand-binding pocket
LPAATβ	Lysophosphatidic acid acyltransferase
LXR	Liver X receptor
MBP	Myristoyl binding pocket
MeOH	Methanol
MS (ESI)	Mass Spectrometry Electrospray ionization
Na_2SO_4	Sodium sulfate
NHR	Nuclear hormone receptor
NMR	Nuclear magnetic resonance
NR	Nuclear receptor
ODCase	Ornithine decarboxylase
PDGF	Platelet derived growth factor
PI_3K	Phosphotidylinositol-3-kinase
PLK 1	Polo like kinase 1
PPAR	Proxisome proliferative-activated receptor
Raf	Proto-oncogene serine/threonine-protein kinase
RAR	Retinoic acid receptors
RET	Rearranged during transfection proto-oncogene
RNA	Ribonucleic acid
RTase	Reverse transcriptase
RXR	Retinoid X receptor
SAR	Structure-Activity Relationships
SERD	Selective estrogen receptor down-regulator

SERM	Selective estrogen receptor modulator
SRC	Steroid receptor coactivator
Src kinase	Sarcoma kinase
TEA	Triethylamine
TKI	Tyrosine kinase inhibitor
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TPase	Thymidine phosphorylase
TR	Thyroid hormone receptor
TrkA	Tropomyocin receptor kinase A
TS	Thymidylate
TSase	Thymidylate synthetase
VEGFR	Vascular endothelial growth-factor receptor
^{13}C-NMR	Carbon nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance

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Chapter 1

Introduction

1.1. General Introduction

According to the American Cancer Society, the most common causes of cancer death in women worldwide are cancers of the lung and bronchus, breast, and colorectum.[1] Each year breast cancer threatens the lives of hundreds of thousands of women and affecting countries at all levels of modernization. Breast cancer begins when cells in the breast's milk-producing glands or in the tubes (ducts) that take milk to the nipples acquire genetic changes that allow them to divide uncontrollably and to move around the body (metastasize). The uncontrolled cell division leads to the formation of a lump that can be detected by mammography (a breast X-ray) or by manual breast examination.[2] Most DNA mutations are acquired rather than being inherited. These acquired mutations of oncogenes and/or tumor suppressor genes may result from environmental, like cancer-causing chemicals or radiation. But so far, the causes of most acquired mutations that could lead to breast cancer are still unknown. [3] Most breast cancers have several acquired gene mutations. Tests to spot acquired gene changes may help doctors more accurately predict the outlook for some women with breast cancer. For example, tests can identify women whose breast cancer cells have too many copies of the *HER2* oncogene. These cancers tend to be more aggressive. At the same time, drugs have been developed that specifically target these cancers and improve outcomes for patients.[4]

Genetic factors such as inherited DNA mutations can dramatically increase the risk for developing certain cancers. For example, there is extensive evidence that mutated *BRCA* genes (*BRCA1* and *BRCA2*), which are tumor suppressor genes, could be inherited and people with such mutants are considered under real risk to develop breast and ovarian cancer.[5] *BRCA* genes are considered "high-penetrance" because they often lead to cancer. Although many of the women with high-penetrance mutations develop cancer, most cases of cancer (including breast cancer) are not caused by this kind of mutation. Women have already begun to benefit from advances in understanding the genetic basis of breast cancer. Genetic testing can identify some women who have inherited

mutations in the *BRCA1* or *BRCA2* (or less commonly in other genes such as *PTEN* or *TP53*). These women can then take steps to reduce their risk of developing breast cancers and to monitor changes in their breasts carefully to find cancer at an earlier, more treatable stage.[6], [7]

Many of the known breast cancer risk factors such as sex, age, family history, early menarche, and late menopause, are not modifiable and cannot be changed. However, other factors associated with increased breast cancer risk, including postmenopausal obesity, use of combined estrogen and progestin menopausal hormones, cigarette smoking, and alcohol consumption are modifiable. Many risk factors affect lifetime exposure of breast tissue to hormones (early menarche, late menopause, obesity, and hormone use). Reproductive hormones are also thought to influence breast cancer risk by increasing cell proliferation, thereby increasing the likelihood of DNA damage, as well as promotion of cancer growth. [3]

Breast cancer is frequently hormone-dependent. That is: hormones stimulate the cancer cells to grow. Vice versa this means that the growth of the cancer cells can be down regulated by the oppositely active hormones or so-called antihormones. Therefore, a hormone therapy (HT) is possible as an adjuvant treatment on breast cancer as well as with metastases. Today surgery, radio-, chemo-, and hormonal therapy form a common combination which has to be coordinated for each individual treatment.[8]

The steroid hormone 17β-estradiol (E) is a key regulator of growth, differentiation, and function in a wide array of target tissues, including the male and female reproductive tracts, mammary gland, and skeletal and cardiovascular systems. It's predominant biological effects are mediated through two distinct intracellular receptors, ERα and ERβ, each encoded by unique genes.[9] Estrogen plays a significant role in the development and growth of hormone-dependent breast cancer.[10] It was reported that approximately 75% of human breast cancers (BCs) are hormone-dependent and are Estrogen Receptor (ERα) positive.[11] Progesterone receptor (PR) and Androgen receptor (AR) are also reported to be expressed in breast cancer and were proposed as potential therapeutic targets in

Estrogen Receptor alpha (ERα) negative breast cancers that retain AR. Patients with (ER) and (PR) positive breast cancer can be treated with hormone therapy (HT) to inhibit ER signaling pathway.[12] HT uses three approaches: (a) antagonizing ER function with antiestrogens such as selective ER modulator (SERM) like tamoxifen; (b) reducing levels of estrogen with aromatase inhibitors (AIs); or (c) down-regulating ER levels with pure antiestrogens.[13] Despite the high level of success of HT, many BCs acquire resistance. The potential mechanisms for either intrinsic or acquired endocrine resistance are still poorly comprehended. Therefore identifying the factors and pathways responsible for this resistance, defining ways to overcome it, and improving treatment options with new therapeutic agents that act in new and alternative pathways are important diagnostic and therapeutic challenges in current breast cancer research and treatment.[14]

Medicinal chemistry plays a central role in discovery of novel anticancer drugs. Pyrimidine either as substituted or fused scaffolds reported in wide range of bioactive moieties including anticancer. The activities of pyrimidine-based anticancer agents is induced through various mechanisms that includes antimetabolites, protein kinase inhibitors such as cycline dependent kinases (CDKs), phosphatidylinositol 3-kinase inhibitors (PI_3K), tyrosine kinase inhibitors (TKIs), or as coactivator binding inhibitors (CBIs**).**[15] **(Table 1.1)** below shows pyrimidine based structures with different anticancer targets. CBIs are small molecules that have the ability to disrupt the protein–protein interaction and inhibit gene transcription in specific receptors like nuclear receptors (NR). The nuclear receptor (NR) includes estrogen receptor (ER), androgen receptor (AR), thyroid hormone receptor (TR), retinoic acid receptors (RAR and RXR) and vitamin D receptor. It is believed that all members of the NR super family evolved from a common ancestor, so there are some similarities in structure and function common to all NRs.[16] The disruption of protein-protein interactions by using CBIs can be used as a method to treat medical conditions including inflammation, Alzheimer's disease, viruses and cancer.[17]

In hormone-dependent breast cancers CBIs that directly disrupt the estrogen or/and androgen receptor/steroid receptor coactivator (SRC) interaction is believed to act as effective alternative approach to inhibit estrogen and androgen signaling and inhibit cancer growth that are resistant to conventional endocrine therapies.[18] Based on a pyrimidine-core system new CBIs would be synthesized and exploited as alternative approach to inhibit estrogen and androgen signaling. These (CBIs) designed to bind in the hydrophobic coactivator groove on the surface of ER and disrupt the interaction between ER and SRC proteins.[19]

Breast cancer remained the most common cancer in women and its incidence continues to rise. Nonetheless, mortality is falling, partly as a result of earlier diagnosis through mammographic screening, improved surgical techniques and attention to margins, improved delivery of radiotherapy, and better adjuvant medical therapies.

Table 1.1: Targets (Enzymes or Receptors) Involved in Anticancer Effects of the Patented Pyrimidines and their Biological Significance.

Class	Target Enzyme/Receptor	Biological Significance
Fused pyrolo pyrimidines	EGFR, HER2, HER3 and HER4 type 1 receptor tyrosine kinases	Suppression of cell death
Fused pyrimidines	Phosphatidylinositol-3 (PI3) kinase	Increases level of phosphatidylinositol-3,4,5-triphosphate (PIP3)
Pyrimidine derivatives	PI3 kinase	Phosphorylation of phosphatidylinositol (PI)
Pyrimidines	Lysophosphatidic acid acyltransferase β (LPAAT β)	Catalyses the acylation of lysophosphatidic acid (LPA) to phosphatidicacid (PA)

Pyrimidines	Nucleoside phosphorylases and Nucleosidases	Inhibition of phosphorylation caused by MTAP and phosphorylytic cleavage of ribo and deoxyribonucleosides catalysed by PNP
Fused pyrrolo pyrimidine	B-Raf V600E, C-Raf, Fms, Kdr,Kit and TrkA kinases	Propagation of biochemical signals in diverse biological pathways
Pyrazolo[3,4d]pyrimidines	EGFR	Phosphorylation of tyrosine amino acid in proteins
2,4,6-Trisubstituted pyrimidines	PI3 kinase	Phosphorylation of phosphatidylinositol (PI)
Heteroalkyl linked pyrimidines	CDK2, FLT3, JAK2 and JAK2V617F	Regulation of cell growth and cell division
Pyrimidines	EphB4, EphA2 (receptor tyrosine kinases)	Phosphorylation of tyrosine residues within an autoinhibitory juxtramembrane region
Pyrimidines	Cdc25A, Cdc25B protein phosphatases	Activation of cyclin-dependent kinase (CDK) by dephosphorylating tyrosine and threonine residues
4-Aryl-2-anilinopyrimidines	PLK	Control of mitotic entry of proliferating cells and regulation of many aspects of mitosis
Substituted pyrimidines	PI3 kinase	Phosphorylation of phosphatidylinositol (PI)

Pyrrolo[2,3-d]pyrimidines	Heat shock protein 90 (Hsp90)	Assistance of protein folding and enhancement of cell survival during stress conditions
Pyrazolo[1,5-c]pyrimidines	Cyclin-dependent kinase (CDK 2,4)	Progression of G1 to S phase of cell cycle and a key component of G1 checkpoint
Fused pyrimidines	AKT	Regulation of transcription, translation, growth and survival
5,7-Disubstituted-3-isopropylpyarazolo[4,3-d]pyrimidines	Platelet derived growth factor (PDGF)	Induction of vascular smooth muscle proliferation
Pyrimidines	ODCase	Catalysis of decarboxylation of OMP to UMP
Cyclopenta[G]quinazolines	Thymidylate synthase (TS)	Catalysis of the methylation of deoxyuridine to thymidine monophosphate
Substituted pyrimidines	Raf-1 kinase, VEGFR-2	Activation of ras signal transduction pathway; Formation of new blood vessels
Pyrimidine indoles	ATR kinase	Regulation of firing of replication origins and repair of damaged replication fork during S phase
Amino pyrimidines	Focal adhesion kinase (FAK)	Transduction of signals transmitted by integrins
4-Morpholinopyrido [3,2-d]pyrimidines	PI3 kinase	Phosphorylation of phosphatidylinositol (PI)

4-Aryl-2-aniliopyrimidines	AuroraB, PLK4, PLK1, PLK2,PLK3, CDK1, CDK4, GSK3b	Chromosome alignment; control of mitotic entry of proliferating cells; regulation of cell division; regulation of nuclear factor Kb nuclear activity
Pyrido[2,3-d]pyrimidin-7-ones	C-Raf, B-Raf	Potential check point for cancer related signal transduction
Pyrrolo[3,4-d]pyrimidines	Abl, Src tyrosine kinase	Phosphorylation of tyrosyl residues of proteins regulate cell growth and differentiation
2,7-Substituted thieno[3,2-d]pyrimidines	Focal adhesion kinase (FAK)	Regulation of migration, proliferation and survival of cells by regulating the signal transduction system of integrin and growth factors
4-(Selenophen-2(or 3)-ylamino)pyrimidine	EGFR	Cell proliferation, survival promotion and apoptosis inhibition
2,4-Diamino-6,7-dihydro-5Hpyrolo[2,3]pyrimidine	FAK/Pyk2 (non receptor tyrosine kinases)	Transduction of signal from a group of stimuli to control cell proliferation, migration and cell survival
Pyrimidines	VEGFR	Phosphorylation of the tyrosine residues in proteins involved in regulation of cell growth, differentiation and survival
Biarylpyrimidines	CDK 9	Cell cycle growth and cellular transcription

Diaminopyrimidines	Hsp90	Assistance of protein folding and enhancement of cell survival during stress conditions
2,4,6-Trisubstituted pyrimidines	Nuclear receptor (NR)	inhibit gene transcription

This table adapted from "Anti-Cancer Pyrimidines in Diverse Scaffolds: A Review of Patent Literature." Recent patents on anti-cancer drug discovery **10** (1): 23-71 Kaur, R., P. Kaur, et al. (2015).[15]

1.2. Chemistry of Pyrimidine

Pyrimidine is a six-member aromatic heterocyclic that composed of four carbon and two nitrogen atoms at positions 1 and 3 of the ring **(Figure 1.1) (2)**.[20] It is isomeric with two other forms of diazines, 1,2-diazine (pyridazine) **(1)**, 1,4-diazine (pyrazine) **(3) (Figure 1.1)**. Pyrimidines as other diazines are important natural constituents and building block with high availability in living organisms. Arguably the most important function of pyrimidines is construction of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).Three nucleobases are pyrimidine-based thymine **(4)**, cytosine **(5)** and uracil **(6) (Figure 1.1)**.[21] The first pyrimidine derivative, alloxan **(7) (Figure 1.1),** was isolated in 1818 by Gaspare Brugnatellilpg by oxidation of uric acid with nitric acid and known for its diabetogenic action in a number of animals. [22, 23]

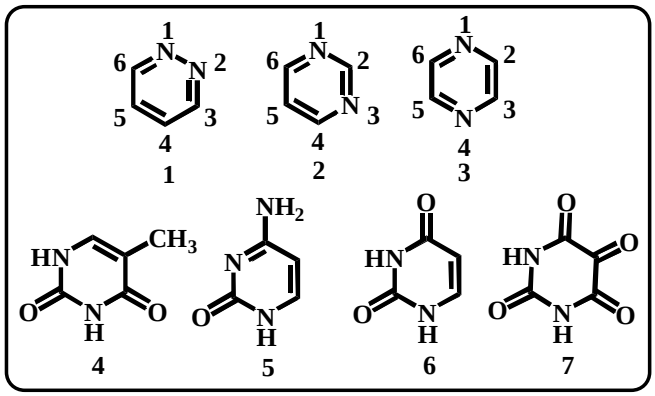


Figure 1.1: Structure of Diazines.pyridazine **(1)**, pyrimidine **(2)**, pyrazine **(3)**; DNA, RNA pyrimidine building blocks: thymine **(4)**, cytosine **(5)**, uracil **(6)** and alloxan **(7)**.

Pyrimidine nucleus constitutes central part of natural products **(Fig 1.2)**. B vitamins thiamine **(8),** riboflavin (**9),** and folic acid **(10)**, antibiotics like bleomycin **(11)**. A variety of natural products such as alkaloids from plants and marine organisms also contain pyrimidine ring system including hypoxanthine **(12)**, xanthine **(13)** found in tea, caffeine **(14)**, theophyline **(15)** constituents of tea leaves and theobromine **(16)** that found in cocoa beans.[24]

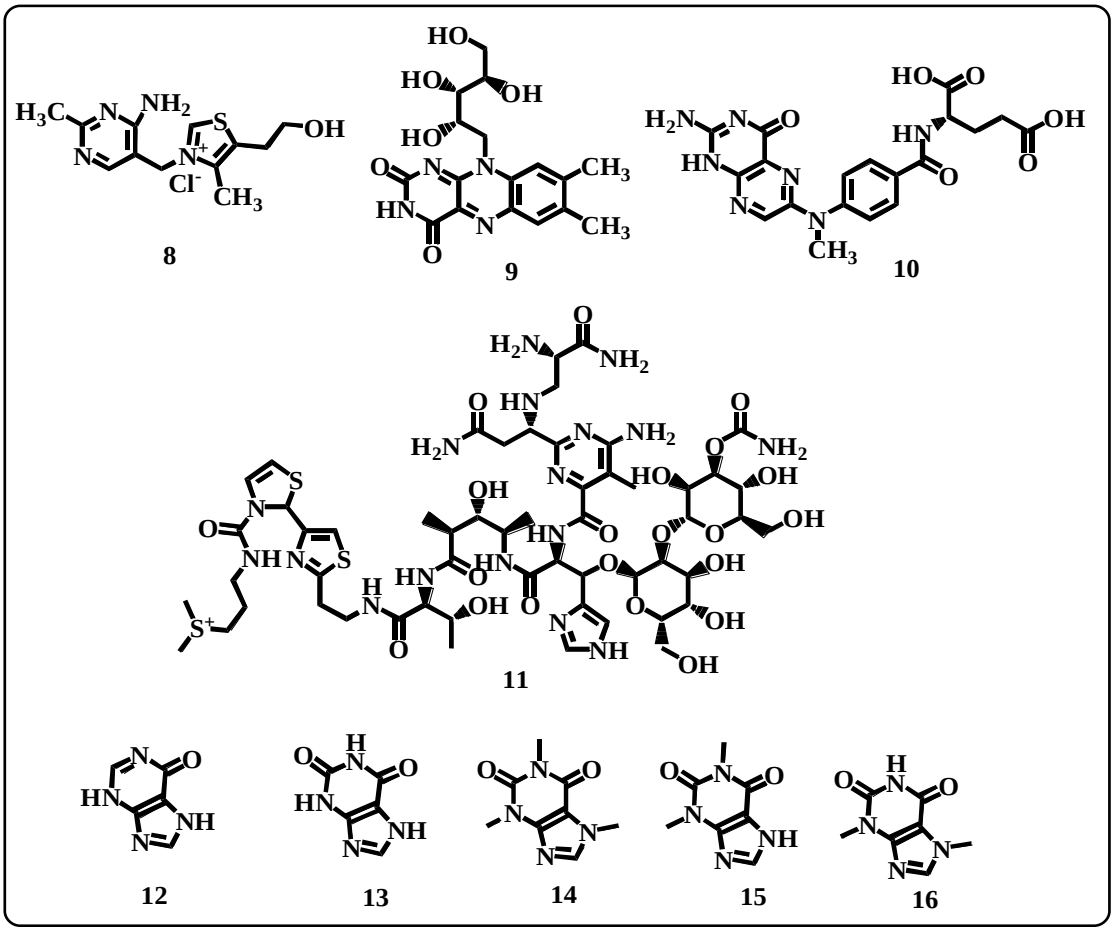


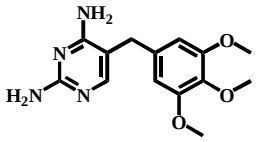
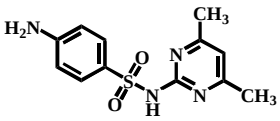
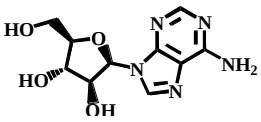
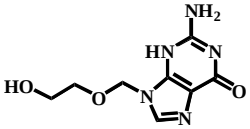
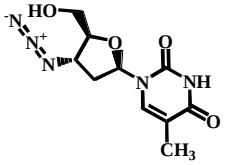
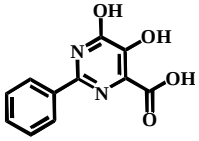
Figure 1.2: Natural compounds containing pyrimidine. Thiamine **(8),** riboflavin (**9),** folic acid **(10)**, bleomycin **(11)**, hypoxanthine **(12)**, xanthine **(13)** caffeine **(14)**, theophyline **(15)**, and theobromine **(16)**.

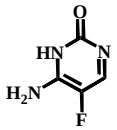
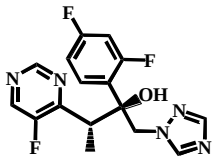
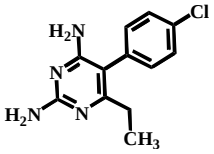
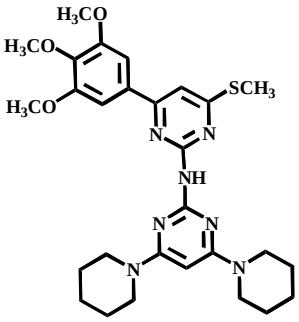
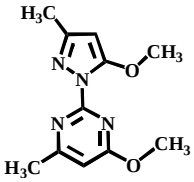
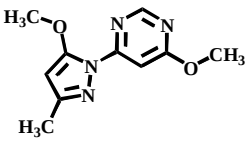
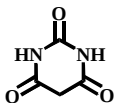
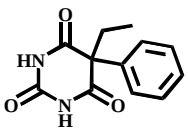
1.3. Therapeutic Potential of Pyrimidines Derivatives

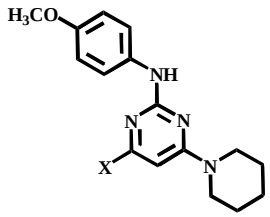
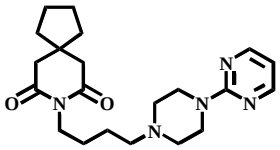
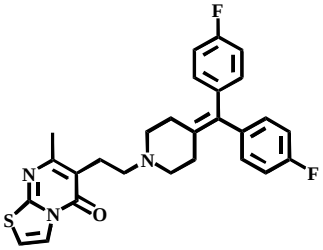
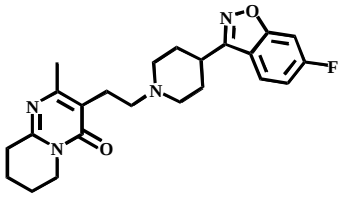
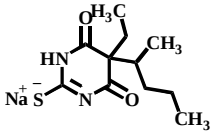
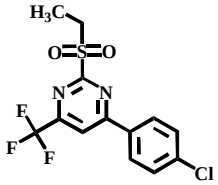
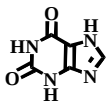
Large array of pyrimidine derivatives possess a variety of pharmacological properties including anticancer, antibacterial, antifungal, antileishmanial, antiviral, anti-inflammatory, analgesic, antihypertensive, antipyretic, antidiabetic, antiallerggic, anticonvulsant, antioxidant, antihistaminic, herbicidal and many of pyrimidines derivatives are reported to possess potential

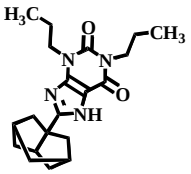
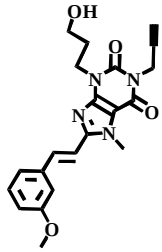
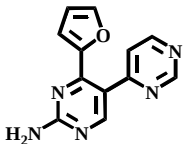
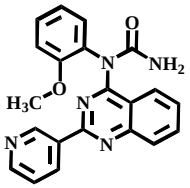
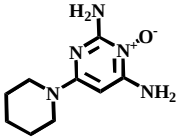
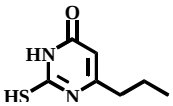
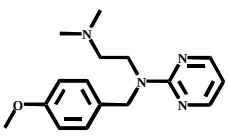
central nervous system (CNS) depressant properties and also act as calcium channel blockers.[25] Given below in **(Table 1.2)** is a brief account of various modifications reported on pyrimidine nucleus, specifically fused and substituted pyrimidines which showed a variety of biological and pharmacological activities.

Table 1.2: Pyrimidine based structures with different biological structures.

Number	**Pyrimidine Derivative**	**Structure**	**Biological Activity**
17	Trimethoprim		Antibacterial (dihydrofolate reductase inhibitor (DHFRI)[26]
18	Sulfadimidine		Sulfonamide antibiotic (Dihydropteroate synthetase inhibitor) [25]
19	Vidarabine		Antiviral activity against Herpes Simplex Viruses (HSV) [27]
20	Acyclovir		Antiviral activity against HSV, and varicella-zoster virus (VZV) [25]
21	Zidovudine		Anti-HIVactivity [25]
22	5,6-Dihydroxy-2-phenylpyrimidine-4-carboxylic acid		Antihepatitis C virus (HCV) activity [28]

23	5-Flucytosine		Antifungal [29]
24	Voriconazole		Antifungal [25]
25	Pyrimethamine		Antimalarial activity (DHFR dihydrofolate reductases inhibitor of malarial plasmodia[30]
26	(4,6-Dipiperidin-1-yl-pyrimidin-2-yl)-[4 methylsulfanyl-6-(3,4,5-trimethoxy-phenyl)pyrimidin-2-yl]-amine		Antileishmanial activity [31]
27	Epirizole		Antipyretic, analgesic, and anti-inflammatory activity (NSAID)[23]
28	Dulcerozine		Anti inflammatory [32]
29	Barbituric acid		Aentral nervous system (CNS) depressants. sedative-hypotic and anticonvulsant [33]
30	Phenobarbital		Aedative-hypotic and anticonvulsant.[33]

31	2,4,6-triamino pyrimidines		Antiepileptic pyrimidines agents[34]
32	Buspirone		Anxiolytic [23]
33	Mezilamine		Antipsychotic agent [23]
34	Piribedil		Antiparkinsonian drug which acts as a dopamine D2 and D3 receptor agonist [23]
35	Sodium thiopental		Rapid-onset short acting gereral anesthetic [33]
36	4-(4-Chloro-phenyl)-2-ethanesulfonyl-6-trifluoromethyl-pyrimidine		Insulinotropic effects (GLP-1 receptor activator) [35]
37	Xanthines		Adenosine Receptor Antagonists[23]

38	(8-(noradamantan-3-yl)-1,3dipropyl xanthine) **{KW-3902}**		Selective adenosine A1 receptor antagonist to treat acute renal failure, hypertension and induce dieresis. It has a partial protective effect against renal vasoconstriction during hypoxemia.[36]
39	3-(3-Hydroxy-propyl)-8-[2-(3-methoxy-phenyl)-vinyl]-7-methyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione **(MSX-2)**		A2A adenosine receptor antagonist [37]
40	2-amino- 4-(2-furanyl)-5-(4-pyrimidinyl)pyrimidine (LAS38096)		A2B adenosine receptor antagonist [37]
41	*N*-(2-methoxyphenyl)-*N*-[2-(3-pyridyl)quinazolin-4-yl]urea		A3 adenosine receptor antagonist [38]
42	Minoxidil		Antihypertensive [23]
43	Propylthiouracil		Antithyroid (antihyperthyroidism)[23]
44	Thonzylamine		Antiallergic H1antihistamine [39]

1.4. Pyrimidines and Pyrimidine Derivatives as Anticancer Agents

Human cells have the capacity to salvage purines and pyrimidines for the synthesis of deoxyribonucleotides that are used for DNA synthesis, and analogues of these nucleotide precursors have proven to be an important class of anticancer agents.[40] There are a total of 14 purine and pyrimidine antimetabolites that are approved by the FDA for the treatment of cancer, which account for nearly 20% of all drugs that are used to treat cancer. Some of the first compounds approved by the FDA for the treatments of cancer were in this class of compounds is 6-mercaptopurine **(45) (Figure1.3)**, that approved for commercial release in September, 1953 for the treatment of childhood leukemia, where it is curative and is still the standard of treatment for this disease. [41] The chemotherapeutic efficacy of pyrimidine derivatives is related to their ability to inhibit vital enzymes responsible for DNA biosynthesis as dihydrofolate reductase (DHFR), thymidylate synthetase (TSase), thymidine phosphorylase (TPase) and reverse transcriptase (RTase). Or inhibit proteins that activate the signal transduction pathway as kinases proteins to act as CDK inhibitors, TNF-α inhibitors, Abl tyrosine protein kinase inhibitors, PI-3 kinase inhibitors, and cytokines inhibitors.[42]

Other pyrimidine-based anticancer drugs in clinical use are shown in (**Figure1.3**). Fused pyrimidine containing such as cladribine **(46)**, clofarabine **(47)**, fludarabine (**48),** gefitinib **(49)**, erlotinib **(50)**, substituted pyrimidines found in capecitabine **(51)**, cytarabine **(52)**, decitabine **(53)** [43], [44] bafetinib **(54)**, imatinib mesylate **(55)** and nilotinib **(56)** are examples of 2,4-disubstitutedpyrimidies that represent as potent tyrosine kinase inhibitors with significant efficacy as first- or second-line treatment in patients with chronic myeloid leukemia. Dasatinib **(57)** which also used for leukemia treatment is 2,4,6-triusubstituted pyrimidine compound. [45]

Due to the great potential of activities of pyrimidines and its derivaties in cancer treatment, we planned to synthesize new various 2,4,6 trisubstituted pyrimidine derivatives and evaluate the

intermediate compounds (2,4-disubstituted pyrimidines) and the final compounds (2,4,6-trisubatituted pyrimidines).

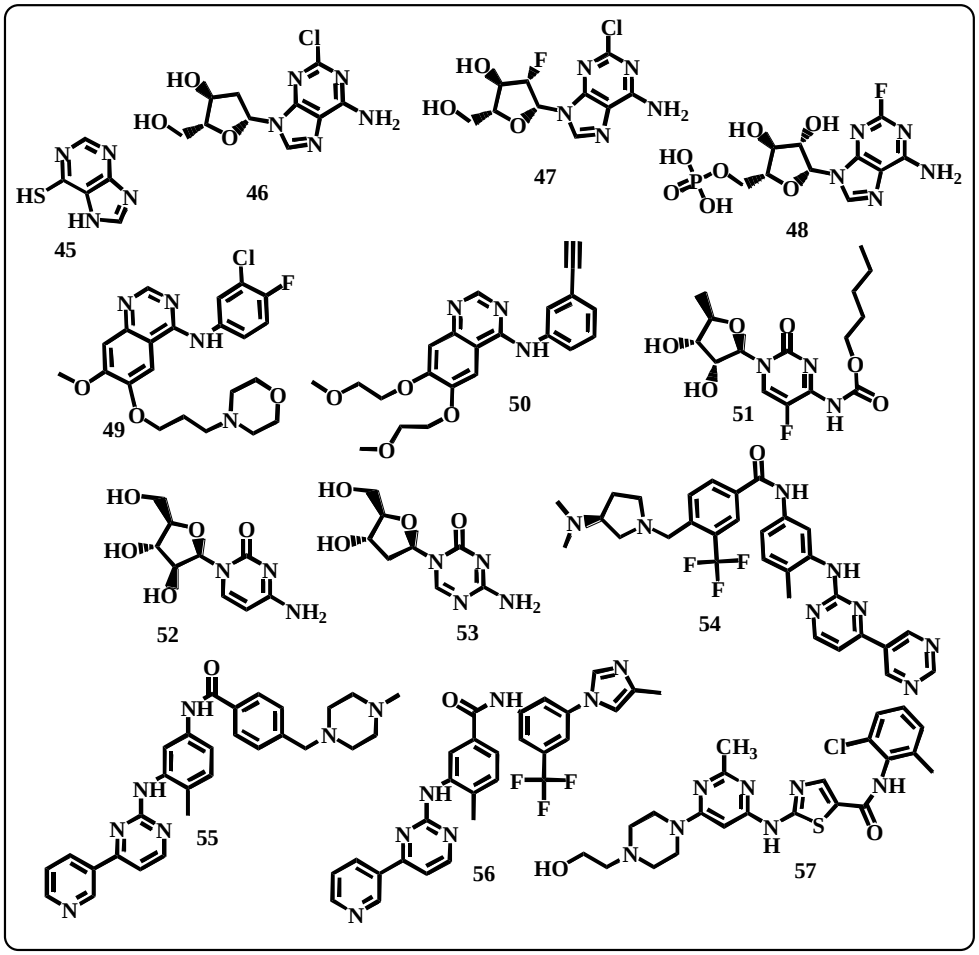


Figure 1.3: potent anticancer agents contain pyrimidine moieties. 6-mercaptopurine **(45)**, cladribine **(46)**, clofarabine **(47)**, fludarabine **(48)**, gefitinib **(49)**, erlotinib **(50)**, capecitabine **(51)**, cytarabine **(52)**, decitabine **(53)**, bafetinib **(54)**, imatinib mesylate **(55)**, nilotinib **(56)** and dasatinib **(57)**.

1.5. Pyrimidine as a Co-activator Binding Inhibitors (CBIs) of Nuclear Receptors (NR)

1.5.1. Nuclear receptor structure and mechanism of action

Nuclear receptors NR are a class of proteins found within cells; they function as ligand-activated transcription factors that regulate the expression of target genes to affect processes as diverse as development, reproduction, and general metabolism.[46] NRs work in concert with coactivators and corepressors to activate and suppress target gene expression.[47] These proteins were first recognized as the mediators of steroid hormone signaling and provided an important link

between transcriptional regulation and physiology. In the mid-1980s, the steroid receptors were cloned and found to exhibit extensive sequence similarity.[46, 48] The subsequent cloning of other receptor genes led to the unexpected discovery that there were many more nuclear receptor–like genes than previously suspected. Today, the human genome is reported to contain 48 members of this transcription factor family.[49], [50] The nuclear receptor superfamily can be divided into three groups of receptors. The first group (class I) includes the steroid receptors (estrogen receptor ER, progesterone receptor PR, androgen receptor AR, glucocorticoid receptor GR, and mineralocorticoid receptor). These steroid receptors bind to DNA as homodimers and recognize palindromic response elements (5'-TGTTCT-3' sequence). In the absence of bound ligand, steroid receptors are coupled to a large multiprotein complex (heat-shock proteins), including Hsp90, Hsp56, Hsp70, and p23. This complex maintains the receptors in a conformation able to bind ligand, and sequesters the receptors to the cytoplasm. In this state, the receptors are unable to influence the rate of transcription of their cognate promoters. Upon ligand binding, the steroid receptors dissociate from the heat-shock proteins Hsps , homodimerize, and translocate to the nucleus, where they bind in a head-to-head arrangement to the DNA response elements.[51],[52] [53] **(Figure1.4 (a)).** The second group of nuclear receptors (class II) includes retinoic acid receptors RAR, thyroid hormone receptors TR, and the vitamin D3 receptor VDR. These receptors are found strictly in the nucleus and form heterodimers with the receptor for 9-cis retinoic acid (RXR) and do not avidly interact with heat-shock proteins Hsps. Some unliganded NRs of this class may interact with DNA and act as transcription repressors. This may be the result of interaction with co-repressor proteins. Hormone binding induced conformational changes which facilitate interactions with coactivator proteins that remodel chromatin, controls polymerase binding and the expression of target genes **(Figure 1.4 (a))**. [54] The third group (class III) consists of orphan receptors, so-called because the endogenous ligands, target genes, and

physiological functions for these proteins are not currently identified. Orphan receptors can function as homodimers or monomers.[52]

The nuclear ligand-regulated receptors have been successful targets for drugs treating a variety of human diseases. Primary examples include estrogen receptor (ER), the target for tamoxifen in breast cancer therapy; glucocorticoid receptor (GR), the target for dexamethasone and prednisolone as anti-inflammatory therapies; and peroxisome proliferator activated receptors (PPARs) such as PPARg, which is the target for rosiglitazone in type 2 diabetes therapy.[55]

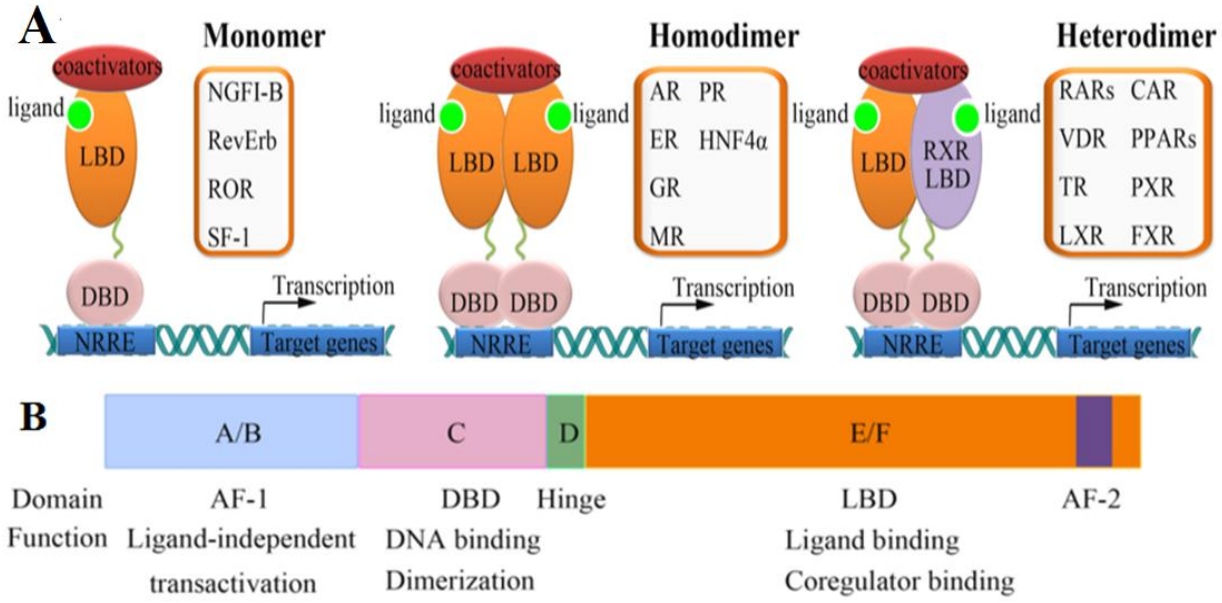


Figure 1.4: Structural and functional organization of nuclear receptors **(a)** Basic mechanism of nuclear receptors. **(b)** Nuclear receptor structure. Adapted from Targeting Nuclear Receptors with Marine Natural Products (Yang, 2014)[56]

Members of the NR superfamily share a similar modular structure with functionally distinct domains **(Figure 1.4 (b)),** including a N-terminal A/B domain includes activation function-1 (AF-1) region and central DNA-binding domain (DBD). However, the primary target for drug discovery is the C-terminal ligand binding domain (LBD), which contains the activation function-2 (AF-2) surface that serves as a binding site for coregulator proteins.[56] The nuclear receptor LBD is the segment of the receptor which changes its conformation upon lipophilic

ligand binding; it is the physiologic binding site for natural ligands such as 17β estradiol (ER), cortisol (GR), and 1a,25-dihydroxyvitamin D3 (vitamin D receptor; VDR).[57] **Table (1. 3)** below contains some types of nuclear receptors with therir ligands and biological roles in the body.

Table 1.3: Some types of nuclear receptors and biological roles in the body.

NR Name	NR Ligand	Role of NR
Estrogen Receptor (ER) ERα and ERβ	17β-estradiol (estrogen (E))	Regulation of growth, differentiation, and function in a wide array of target tissues, including the male and female reproductive tracts, mammary gland, and skeletal and cardiovascular systems.[58]
Progesterone Receptor (PR) PR-A and PR-B	Progesterone	Preparing the uterus endometrium for embryo implantation. It also suppresses milk protein synthesis in the mammary gland during pregnancy and regulates the outgrowth of alveolar structures in the mammary gland.[59]
Androgen Receptor (AR)	Testosterone & 5α-dihydrotestosterone (DHT)	Differentiation of the reproductive organs into the male phenotype, induces the secondary sexual characteristics, maintaine male skeletal integrity, development & maintenance of normal prostate.[53, 60]
Glucocorticoids Receptor (GR)	Glucocorticoids (cortisol)	Regulation of glucose metabolism (gluconeogenesis), inhibition of bone formation, anti-inflammatory and immunosuppressive actions.[61]
Mineralocorticoids Receptor (MR)	Aldosterone	control sodium reabsorption and blood pressure.[61]
Retinoic acid receptors (RARs) Retinoid X receptors (RXRs).	Retinoic acid	Essential for reproduction, embryonic development, epithelial differentiation, immune function, vision and homeostasis. Regulate of epidermal cell growth and differentiation.[62]

Vitamin D receptor (VDR)	Calcitriol (1,25-dihydroxyvitamine)	Essential role in mineral metabolism, skeletal health and immunity, cell proliferation and differentiation. [63]
Peroxisome proliferator-activated receptors (PPARs) PPARα, PPARδ/β, and PPARγ	Thiazolidinedione	Regulate lipid metabolism, glucose homeostasis, cellular differentiation, and inflammatory response.[64]
Liver X receptors (LXRs) LXRα and LXRβ	Oxysterols	Lipid metabolism, including cholesterol and fatty acids homeostasis; steroidogenesis; glucose homeostasis; inflammation and immunity,[65] antidiabetic properties through suppression of hepatic gluconeogenesis and enhance peripheral glucose uptake.[66]
Estrogen-Related Receptors (ERRs) ERRα, ERRβ, and ERRγ	Orphan NR No identifide ligand	Regulating cellular metabolism through the regulation of genes involved in glycolysis, oxidative phosphorylation, and tricarboxylic acid cycle.[67]
Chicken Ovalbumin Upstream Promoter Transcription Factors (COUP-TFs) COUP-TFI and COUP-TFII	Orphan NR No identifide ligand	Regulation of several important biological processes, such as neurogenesis, organogenesis, cell fate determination, and metabolic homeostasis.[68]
Nerve growth factor IB-like receptor (NGFI-B)	Orphan NR No identifide ligand	Controling of inflammation, proliferation, apoptosis, thrombosis, and angiogenesis.[69]
‘‘Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1’’ (DAX1)	Orphan NR No identifide ligand	Regulation of development and function of both the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal axes. Act as an anti-Sry factor in the process of gonadal sex differentiation.[70]

1.5.2. Nuclear receptor coregulator

Transcriptional coregulators have been revealed as the principal regulators of gene expression by directly interacting with and modulating the activity of essentially all nuclear receptors (NRs) and transcription factors. [52] On the basis of their functional output, coregulators can be sorted into two

major classes: coactivators, which are associated with agonist-bound NRs to induce gene expression, and corepressors, that selectively repress gene expression through interaction with unliganded or antagonist-bound NRs. [9] [71] Transcriptional induction is an ordered and dynamic process in which a NR first receives a signal from its cognate ligand and then translocates to specific DNA sequences in the promoter region of a target gene. [72] This is followed by the recruitment of coactivator complexes, which then mediate enzymatic reactions, remodeling the chromatin and facilitating the association of the RNA polymerase II complex with the general transcriptional machinery at the target gene promoter. Because coregulators impact the transcriptional activity of multiple NRs and other types of transcription factors, they exert broad genome-wide effects on genetic networks and contribute significantly to a large spectrum of physiological abnormalities and diseases **(Figure 15)**.[73]

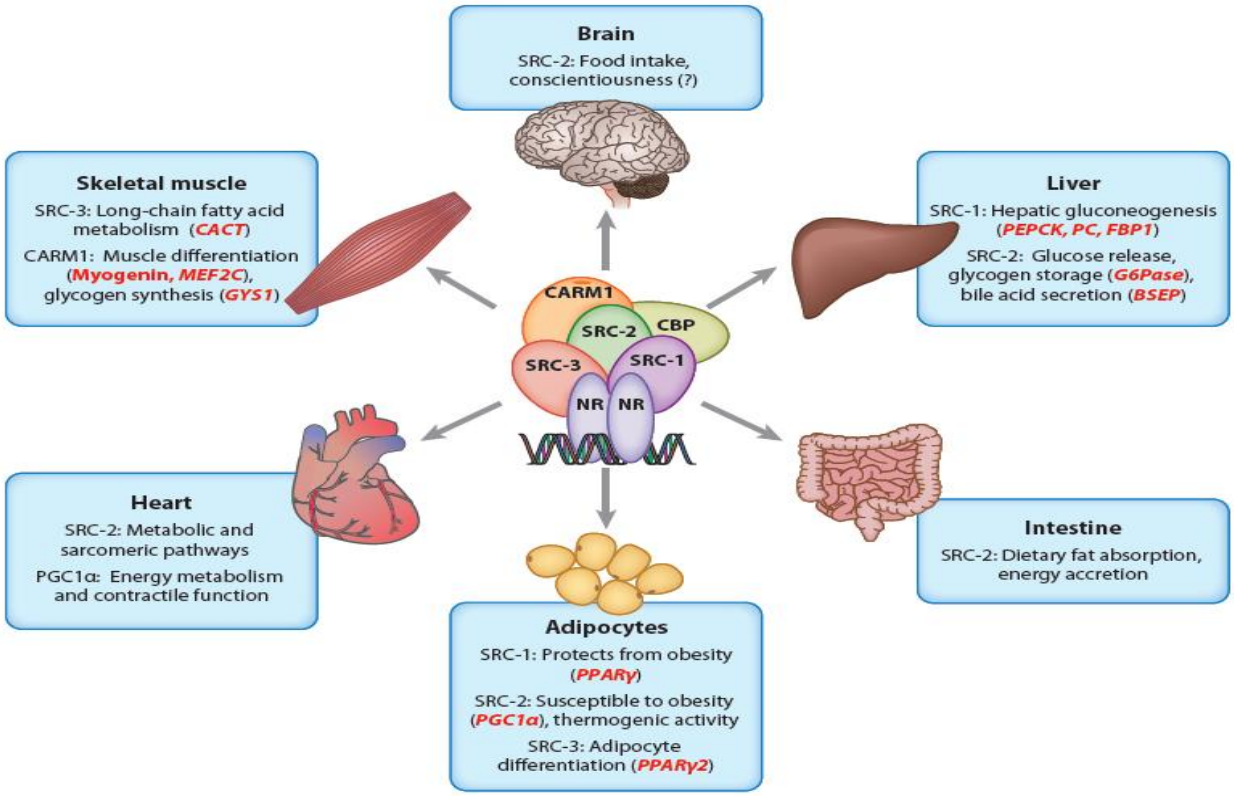


Figure 1.5: Nuclear receptor coactivators control the physiology of multiple organs that are critical for various metabolic disorders and some rare genetic diseases as well. Target genes transcriptionally regulated by the coactivators that indicated in red. Adapted from Dasgupta *et.al* [73]

SRC family of the three members (SRC-1, SRC-2, and SRC-3) of the p160 class of coactivators have been widely implicated in the regulation of steroid hormone action by mediating functions of a majority of the NRs and transcription factors, and their abnormal expression in different malignancies and genetic diseases has distinguished them as master regulators of human pathologies[74] **(Figure 1.6)**. Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α) is another important coactivator that involved in the regulation of metabolism and energy homeostasis. Expression levels of PGC-1α in various tissues have been associated with genetic predispositions to diseases with impaired mitochondrial function, including type 2 diabetes mellitus and obesity.[75] Other coactivators, such as CREB-binding protein (CBP)-p300, interact with virtually all transcription factors and regulate gene expression by relaxing chromatin structure at the target gene promoter through their intrinsic histone acetyltransferase (HAT) activity. In addition to chromatin remodeling, coactivator-associated arginine methyltransferases (CARM1/PRMT4) also stimulate gene transcription by activation of NRs and other transcription factors in combination with the SRC family of coactivators.[73]

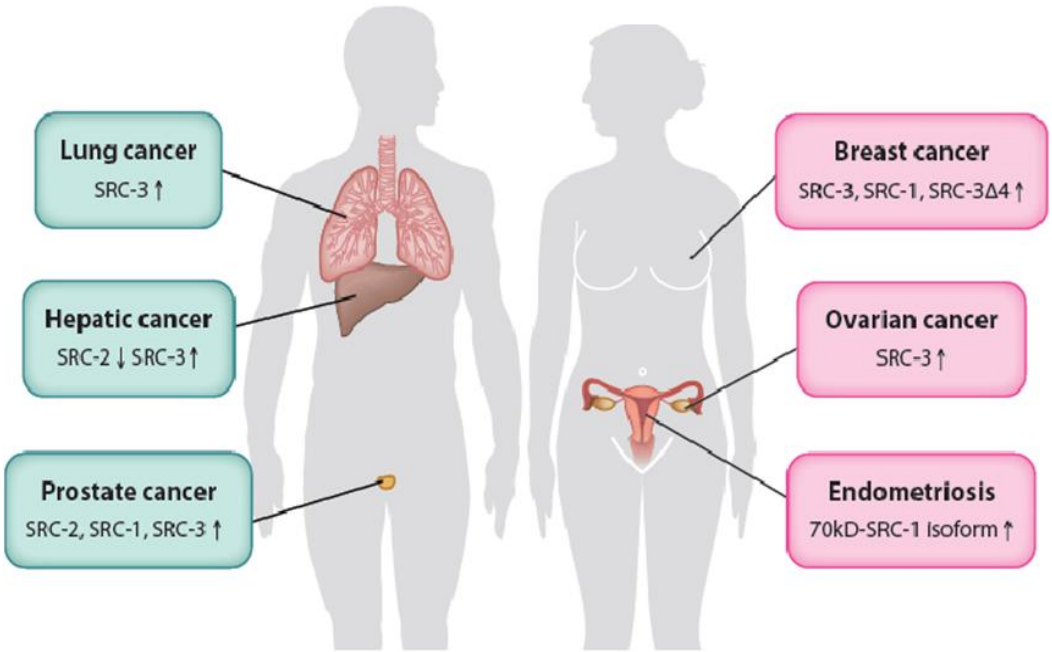


Figure 1.6: Steroid receptor coactivators (SRC family members) are abundantly expressed and amplified in various forms of cancer. Adapted from Dasgupta *et.al* [73]

NR coactivators are unable to bind directly to the DNA. Instead they form multiple contacts with the NR and with each other in multi-protein cooperative coactivator complexes. Initial investigations into coactivator complexes reported that steady-state SRC complexes consist of six to ten stably associated proteins and many more loosely-bound proteins.[76]

SRCs contain three structural domains. The N-terminal basic helix-loop-helix-Per/ARNT/Sim (bHLH-PAS) domain is the most conserved region and is required for protein-protein interactions. The bHLH-PAS domain can interact with several transcription factors such as myogenin, MEF-2C and TEF to potentiate transcription. The central region contains three LXXLL (L for leucine and X for any amino acid) motifs, which form amphipathic α-helices and are responsible for interacting with NRs. The C-terminus contains two transcriptional activation domains (AD1 and AD2). AD1 binds CBP (CREB (cAMP response element-binding protein)) and p300 histone acetyltransferase (HAT), and the recruitment of CBP or p300 by SRCs to the chromatin context is essential for SRC-mediated transcriptional activation. AD2 interacts with coactivator-associated arginine methyltransferase 1 (CARM1) and protein arginine methyltransferases (PRMT1), which are histone methyltransferases. The C-termini of SRC-1 and SRC-3 contain HAT activity domains, although their cellular substrates are incompletely identified. These molecular features provide SRCs with a suitable structural base for recruiting additional coregulators and general transcription factors, which in turn results in chromatin remodeling, assembly of general transcription factors and recruitment of RNA Polymerase II for transcriptional activation. The basic SRC structural domains and the simplified functional mechanisms for SRCs in NR-dependent transcriptional activation are sketched in **(Figure1.7).**[77] To exert their coactivation function in transcription, SRC interact with hormone (H)-bound nuclear receptors (NRs) to recruit other components of a large coactivator complex to the hormone response elements of a target gene. Specifically, SRCs bind NRs through one of their three LXXLL motifs (L1, L2 and L3) in the NR interaction domain (NRID) and interact with CBP and p300 through their activation

domain 1 (AD1), with CARM1 and PRMT1 through their AD2 and with SWI/SNF through AD3. p/CAF is a p300/CBP-associated factor. CBP, p300 and p/CAF are histone acetyltransferases. CARM1 and PRMT1 are histone methyltransferases. RHA is a RNA helicase. SWI/SNF is an ATP-dependent chromatin remodeling complex. The formation of such a coactivator complex results in chromatin remodeling and bridges the hormone-activated NRs with the general transcription machinery for transcriptional activation of their specific target genes.[77]

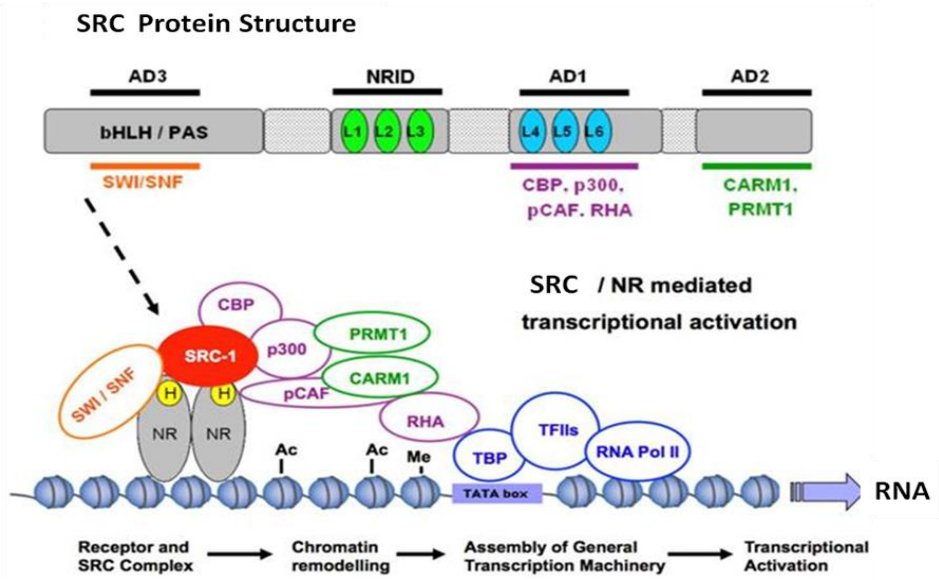


Figure 1.7: Molecular structure of SRCs and their functional mechanisms in steroid hormone-induced gene expression .Adapted from Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family (Xu *et.al*, 2009). [77]

1.5.3. Conformational changes in NRs ligand binding domaine (LBD)

Ligand binding domain (LBD) adopts a three-layer antiparallel α-helical sandwich fold, consisting of 12 *α*-helices with a ligand-binding pocket. The hydrophobic AF-2 surface (helix 3/4/5/12 interface) provides a binding site for coactivator proteins with an LXXLL recognition motif.[78] Conformational changes happen in the LBD upon binding agonists, antagonists, or partial agonists. These conformational changes vary depending on which particular type of molecule is bound and govern the nuclear receptor's ability or inability to activate gene transcription. The most striking difference in the conformation of the LBD structures is the orientation of helix H12, which contains the residues of the AF-2 activation domain core. In the apo or unliganded

conformation (**Figure1.8 (a))**, helix 12 is generally thought to be extended away from the LBD.[79] Upon agonist ligands binding, this helix rotates nearly 180° to pack tightly against the LBP, serving as a lid closing the entrance to the LBP (**Figure1.8(b))**. This brings the AF-2 into position to generate a surface for binding of coactivators that are necessary for nuclear receptor gene transcription activation. [55]

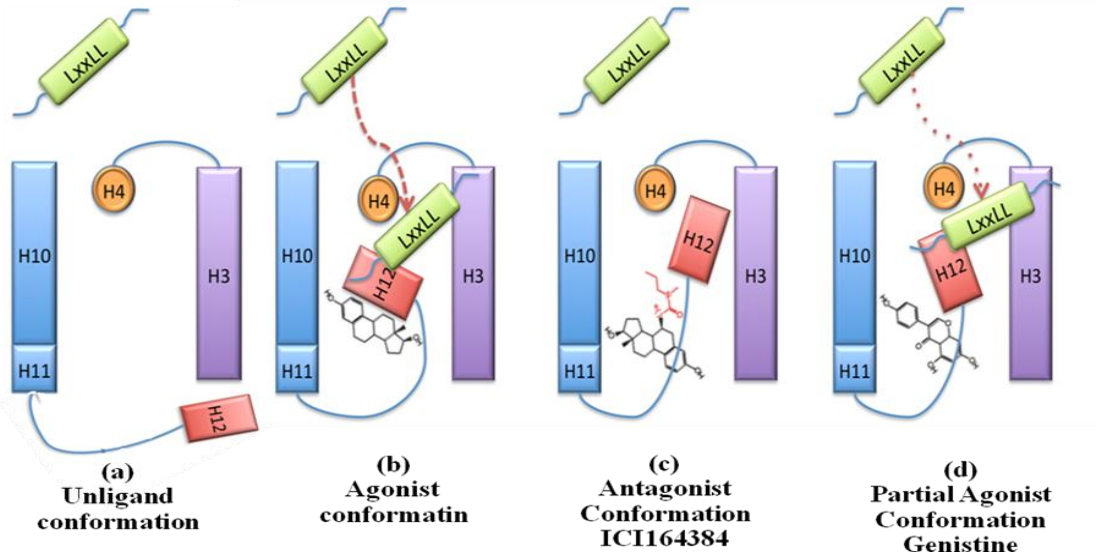


Figure 1.8: Ligand binding to NRs results in a conformational change in the protein. **(a)** apo conformation, **(b)** agonist conformation, **(c)** antagonist conformation, **(d)** partial conformation. Adapted from Nuclear Hormone Receptors: A Brief Overview J.P. Vanden Heuvel, INDIGO Biosciences Inc., State College PA. (Heuve, 2009)[41]

Antagonist binding inhibits gene transcription. Unlike agonists, they have bulky side chains that cannot be accommodated within the agonist binding cavity. This bulky side chains sterically prevent the positioning of helix H12 in the active conformation and no coactivator interface is formed (**Figure 1.8 (c))**. The antagonists also induce unwinding of the C-terminal part of helix H11. This allows helix H12 to bind to the coactivator binding region, therefore blocking coactivator binding. In contrast, agonists stabilize the long H11 helical conformation.[80] Pure antagonists differ from partial agonists based largely in their steric properties. Partial agonists do not contain a bulky extension, so they do not sterically preclude the agonist position of H12 (**Figure1.8 (d))**. In this respect, they are more like agonists. However, these molecules do induce unwinding of helix H11 and in this respect are similar to antagonists. The partial activity of the

ligand in this case is attributed to a weak stabilization of the active position of H12, so the active conformation of the LBDs is not stabilized. The position of H12 in this case probably depends on the intracellular concentration of co-activators and co-repressors.[55] These ligand-bound structural observations have been used as a model to understand the mechanism of action of ligands and are generally used as a guide for nuclear receptor virtual ligand screening and structure-based drug design efforts.

Of all the surfaces available on a given NR, the singular site for regulation of receptor activity has almost invariably been the ligand-binding pocket of the receptor, the site where agonists, antagonists, and selective NR modulators interact. With the increasing understanding of the multiple molecular components involved in NR action, researchers have recently begun to look to additional interaction sites on NRs for regulating their activities by novel mechanisms. The alternate NR-associated interaction sites that have been targeted include the coactivator-binding groove and allosteric sites in the ligand-binding domain, the zinc fingers of the DNA-binding domain, and the NR response element in DNA. [81] Phenotypic and conformation-based screens have also identified small molecule modulators that are believed to function through the NRs and act as direct protein/ protein disruptors of the interaction between the nuclear receptor (NR) ligand binding domain (LBD) and the receptor coactivators. These compounds were termed as coactivator binding inhibitors or (CBIs).[82] CBIs have been discovered for the estrogen receptor (ER), thyroid receptor (TR), androgen receptor (AR), and the peroxisome proliferator activated receptor (PPAR). [83]

1.5.4. Estrogen Receptor and its Role in breast Cancer Biology

The estrogen receptor (ER), a member of the superfamily of ligand-regulated nuclear transcription factors, mediates the action of estrogens, including the primary endogenous ligand 17β-estradiol (E2) (**Figure 1.9**) **(a)**.[84] ERs exist as two subtypes, ERα and ERβ, and because they are found in both reproductive (uterus, ovary, and breast) and non-reproductive (bone, brain, and the

cardiovascular system) tissues, they have emerged as attractive therapeutic targets for the treatment of breast cancer, the prevention of osteoporosis, and the mitigation of menopausal symptoms via hormone replacement.[85] The genes of ERα and ERβ also differ in their location in the genome, as the former resides on chromosome 6 and the latter on chromosome 14. (**Figure 1.9**) **(b)** Shows the genomic composition of ER subtypes.

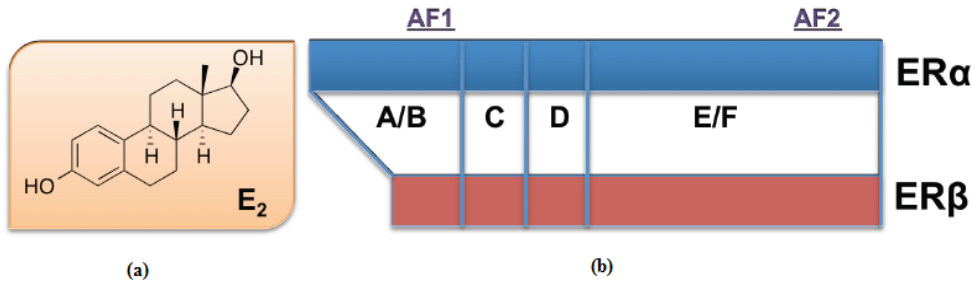


Figure 1.9: **(a)** Estradiol (E_2) structure, **(b)** the genomic composition of ER subtypes. Adapted from Pearce et al. [86]

In contrast to the widely acknowledged paradigms involving chemicals and viruses as tumor initiators and promoters, the hormone-related cancers, such as breast cancer, share a quite different mechanism of carcinogenesis. Hormones, both endogenous and exogenous, by driving cell proliferation, increase the number of cell divisions and the opportunity for random genetic errors. Based on data from both clinical and animal studies, risk factors associated with breast cancer reflect cumulative exposure of the breast epithelium to estrogens. Estrogens are implicated in the development of breast cancer, which induce physiological activities mainly via estrogen receptors (ERs).[87]

Clinical observations and laboratory studies suggest that ER signaling pathway is the major driver in promoting proliferation, survival and invasion of ER-positive breast cancer cells.[88]The expression level of ERα is high and have been directly correlated with an increased risk of breast cancer while it is low in normal breast tissue.[89] Endocrine therapy is the mainstay of treatment for patients with ER-positive breast cancer, especially those with metastatic disease. These therapies include treatments which target ER by blocking receptor binding with an antagonist or by depriving

the tumor of estrogen. The three broad groups of currently approved anti-estrogen therapies are selective estrogen receptor modulators (SERMs) such as tamoxifen **(58)**, toremifene **(59)** and raloxifene **(60)**, which block activity of ER and functions as estrogen antagonist in breast cancer cells, but it also functions as an agonist in some tissues including the bone, uterus, liver, and the cardiovascular system; selective estrogen receptor down regulators (SERDs) such as fulvestrant (ICI 182, 780) **(61)**, which induce destabilization and degradation of ER; [90] and aromatase inhibitors (AIs), including steroidal/irreversible (anastrozole **(62)** and letrozole **(63)**) and nonsteroidal/reversible (exemestane **(64)**) inhibitors **(Figure 1.10)**, which decrease estrogen production in peripheral tissues and within the tumors through inhibition of the enzyme aromatase.[91] Endocrine therapy as the first targeted therapy in cancer treatment has successfully improved outcomes and was beneficial to millions of breast cancer patients in the past 30 years. Nonetheless, finding ways to inhibit ER activation is necessary and is hoped to maximize treatment opportunities in breast cancer treatment [92]

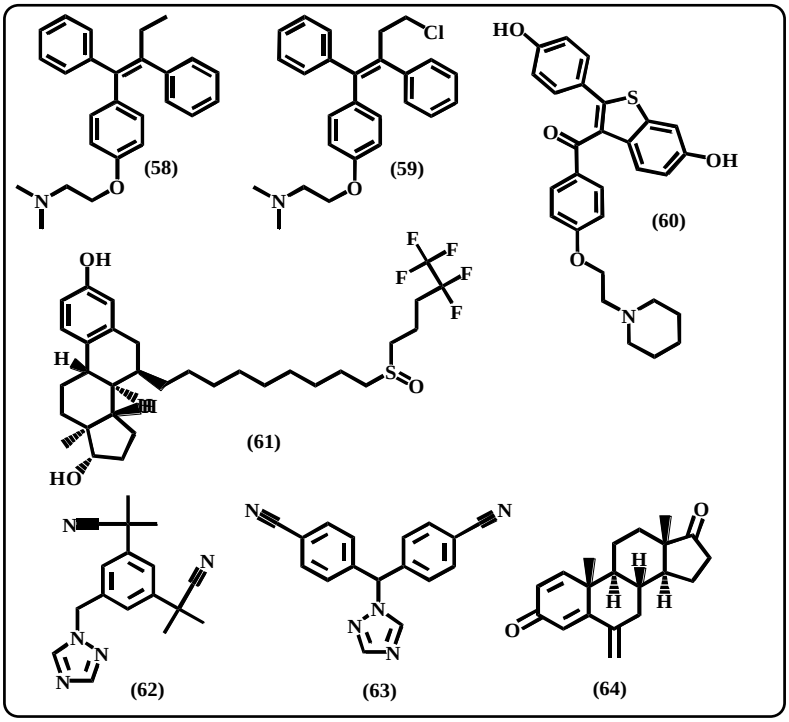


Figure 1.10: anti-estrogen compounds. Tamoxifen **(58)**, toremifene **(59)** and raloxifene **(60)**, fulvestrant **(61)**, anastrozole **(62)**, letrozole **(63), and** exemestane **(64).**

Tamoxifen is the most commonly used treatment for patients with estrogen-receptor (ER)-positive breast cancer as a part of adjuvant chemo-endocrine therapy.[93] Although many patients get benefits from tamoxifen in the adjuvant and metastatic settings, resistance is an important clinical problem. Such mechanisms may include changes in the expression of ERα or ERβ, alterations in co-regulatory proteins, and the influences of cellular kinase signal transduction pathways.[94]

Several different mechanisms have been hypothesized to be involved in the resistance of breast cancer cells to hormonal therapy **(Table 1.4).** About 20% of patients treated with endocrine therapy suffering from ER loss in the tumor over time. Such tumors would no longer be driven by estrogen, but the escape pathways which take over with loss of estrogen dependence still not well defined. Up regulation of HER2 (Human Epidermal Growth Factor Receptor Type 2) by either acquisition of gene amplification or overexpression that occur in some tumors. HER2 may subsequently assume the driving role in tumor progression by serving as an alternative survival pathway or by reducing the level of ER, thus rendering the tumor less responsive to estrogen. Preclinical and clinical data suggest the possibility that tumors can alternate between ER and HER2 as the dominant pathway, with targeted therapy against one pathway causing reactivation of the other. On the other hand, PR is lost more frequently than ER with intervening endocrine therapy and with this loss the tumor becomes more aggressive and patients have a worse survival outcome than patients who maintain PR expression after resistance to one endocrine therapy. In this case, PR loss might be associated with increased growth factor signaling and upregulation of the PI3K pathway, which downregulates PR and ER expression. [95],[96],[97]

Table 1.4: Potential mechanisms of resistance to endocrine therapy in breast carcinoma.

Loss of expression or altered function (mutations) of ERα
Upregulation of HER2 in some patients after endocrine therapy
Loss of PR after progression on endocrine therapy
Metabolism of hormonal agents (CYP2D6 variants for tamoxifen)

Altered expression of co-regulators
Adaptation to estrogen withdrawal
Shorter response duration and less frequent responses with sequential endocrine therapies
Increased growth factor signaling
Eventual loss of dependence on estrogen with resistance to all endocrine therapies

ER has two sites that are prone to be affected by drugs - the Ligand Binding Domain (LBD) in which the natural agonist, Estradiol (E2) resides and affects movement to the cell's nucleus for transcription, and the Coactivator Binding Domain (CBD) which is activated by the binding of E_2, where proteins of the Steroid-Receptor-Coactivator family (SRC-1,2 and 3) bind to ER and help lead it to the nucleus and transcription promotion.

1.5.5. Coactivator Binding Inhibitors (CBIs) of estrogen receptor ER

Traditionally, the inhibition of ER activity has been achieved using antagonist molecules that bind to the ligand binding pocket in place of estradiol and trigger a conformational change that indirectly blocks coactivator binding by an intra-receptor or allosteric process.[98]An alternative and yet underexploited approach to blocking estrogen action involves small molecules that disrupt the interaction between agonist-activated ER and the SRC by an extra-receptor or direct competitive process. Such molecules are termed coactivator binding inhibitors (CBIs). **(Figure 1.11 (I))**.[99]
Compounds termed as coactivator binding inhibitors (CBIs) designed to bind only to the coactivator binding surface of ER by fitting directly into the coactivator binding groove on the surface of the ligand binding domain LBD. Thus, block coactivator recruitment to the estrogen receptor ligand binding domain LBD.[100] [100] The steroid receptor coactivators (SRCs), possess multiple copies of a conserved signature sequence motif, LXXLL (L is leucine and X is any amino acid), known as a nuclear-receptor interaction box (NR-box). The coactivators bind to the nuclear receptor LBD

through a two-turn amphipathic α-helical motif encompassing the NR box LXXLL signature sequence, with the ER-coactivator complex being further stabilized by interactions between the intrinsic dipole moment of the helical coactivator peptide backbone and charged residues (the glutamic acid and the lysine) from the ER at either end of the binding groove. The X-ray structure of the ERα complex with the NR box of SRC shows this interaction in detail **(Figure 1.11 (II))**.[101] Among the key interactions of the ILXXLL sequence are the hydrophobic contacts of the sidechains of the leucine and isoleucine residues with the hydrophobic coactivator binding surface of ER. The isoamyl sidechains of two leucines (ILXXLL) are pointed down towards ER into a deep hydrophobic groove. The side chains of the isoleucine and another leucine (ILXXLL) are sitting on a "shelf above the groove and form a weaker, but essential hydrophobic interaction. [102] The peptide is amphipathic, and the hydrophobic residues on one face of the helix are favorably disposed to project into a non-polar groove on the receptor while the polar side chains of the XX residues, are on the opposite face of the helix are exposed to and can hydrogen bond with water. Also, the inherent dipole of the a-helical peptide orients the NR Box sequence in one direction. A "charge clamp" is present through which a lysine and a glutamic acid on the ER surface can interact favorably with the negative and positive ends, respectively, of the inherent dipole of the helical peptide. [103]

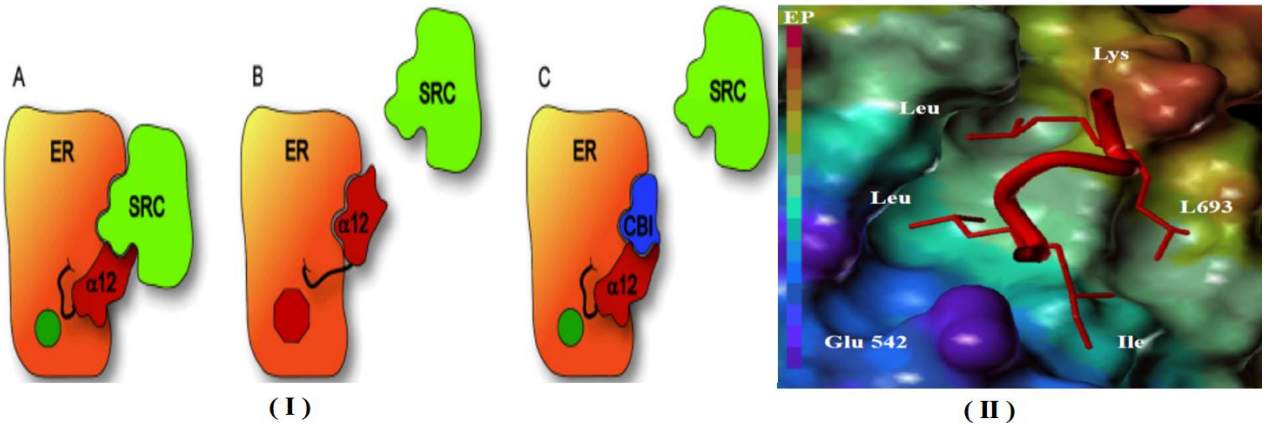


Figure 1.11: (I) electrostatic surface of the estrogen receptor with the coactivator peptide (red tube). **(II)** Cartoon representation of classical vs. CBI antagonism of ER. **(A)** Conformation of agonist-bound ER with helix 12 forming part of the steroid receptor coactivator (SRC) binding site; **(B)**. Conformation of antagonist-bound ER in which helix 12 occupies the SRC binding site,

disrupting the ER/SRC interaction indirectly; **(C**) conformation of agonist-bound ER in which a CBI occupies the SRC binding site, disrupting ER/SRC interaction directly. Adapted from (Parent, 2008) [104].

1.5.6. Literature Reviews

Although it is a recently developed area of research, some CBIs have already been discovered to inhibit ERα -coactivator association. In 2003 Leduc *et al.* created one of the first successful CBIs by mimicking the LXXLL coactivator peptide. They found it was necessary to keep the α-helical character so that the hydrophobic side chains could interact with the pocket, and the two XX hydrophilic residues would face away from the surface as in the natural peptide. After considering these elements, PERM-1, synthesized by Leduc *et al*. showed the best results. This molecule contained a disulfide bridge, to maintain the desired α- helical shape **(Figure 1.12 (a))**. Using a time-resolved fluorescence-based coactivator interaction assay, they were able to observe some selectivity as PERM-1 gave a *K*i of 25 nM and 390 nM, for ERα and ERβ respectively. They were also able to confirm that PERM-1 was binding to the correct site based on an X-ray crystal structure of ER with estradiol and PERM-1.[105]

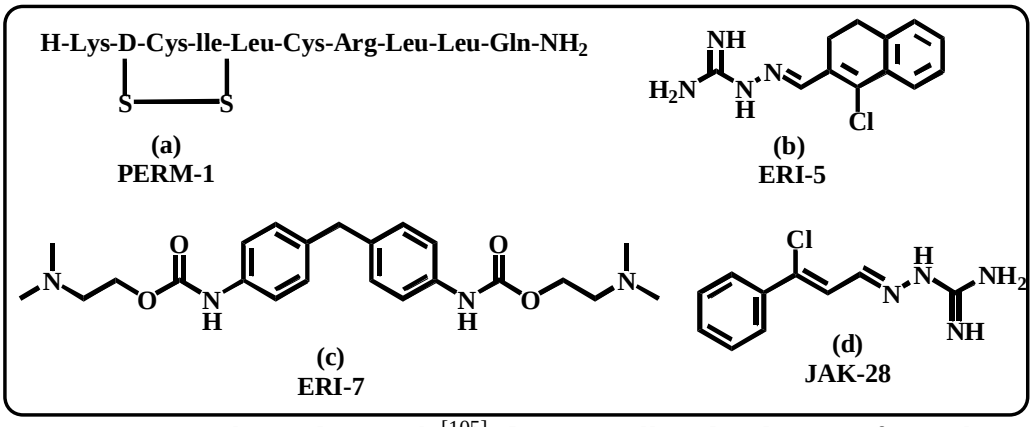


Figure 1.12: **(a)** PERM-1 by Leduc *et al.* [105] **(b)(c)** Small molecule CBIs from Shao *et al.* [106], **(d)** guanylhydrazone-based CBI by Andrew L. LaFrate *et al.*. [107]

Shao *et al.* (2004) took a different approach in the development of a good small molecule ER coactivator inhibitors based on the guanylhydrazone structure. By examining libraries of small molecules via a high-throughput screening (HTS) and virtual screening (VS) for their ability to act

as ERα antagonists. Two successful CBIs were identified, ERI-5 **(Figure 1.12 (b))** was the first hit discovered by HTS that gave an IC_{50} of 5.5 μM in a mammalian two hybrid (M2H) assay, where both estradiol and ERα antagonist (fulvestrant) ICI182,780 **(61) (Figure 1.10**) are present along with steroid receptor coactivator-3 (SRC-3) and human ERα. Further biological testing of ERI-5, in a non cell-based interaction assay containing only SRC and ERα (SEIA), showed that it inhibits ERα-coactivator association. ERI-5 also demonstrated inhibition activity for ERβ. The second small molecule, ERI-7 **(Figure 1.12 (c))** was identified by virtual screening on diethylstilbestrol-bound ERα co-crystallized with an LXXLL peptide. Because it was not as cell-permeable as ERI-5, ERI-7 was tested only in the SEIA assay, and displayed an IC_{50} of 25 μM. Both CBIs were assessed in a competition assay, where it was concluded that they did not displace E2 and were not directly competing with estradiol, but still blocked ERα activity. An NMR assay of ERI-5 and ERI-7 bound to ERα protein showed levels of free estrogen that did not increase when more ERI-5 and ERI-7 was added, meaning there was no estradiol displacement ERI-5 and ERI-7 bound elsewhere on ERα. [106]

Andrew L. LaFrate and his colleagues at the University of Illinois Urbana-Champaign expanded upon this work by designing a synthetic route to these guanylhyrazone-based molecules, starting from α-tetralone.[107] They synthesized a series of 16 potential CBIs and subjected them to mammalian two-hybrid assays and cell-based reporter gene assays to determine their potency for ERα. These studies demonstrated that the compounds do not compete directly with E2 at the LBP. The compound **JAK-28** in **(Figure 1.12 (d))** exhibited the best results with an IC_{50} of 0.9 μM in the cell based reporter gene assay showing that the phenyl group and chlorine are important to binding and that an acyclic middle portion is preferred. Substitutions on the ring proved to be unfavorable. This class of CBIs is interesting because they do not represent the typical α-helical mimic properties, but clearly can adopt of the proper configuration for inhibiting co-activator proteins. [107]

In 2007 the Katzenellenbogen group has also published a study of ERα CBIs that based on a bicyclo[2.2.2]octane. This time they took what they referred to as an "inside-out" approach in which they mimicked the first and third leucine residues of the NR box that extend "inside" the CBP. They then attached structural elements that represent the backbone of the coactivator α-helix which is "out" of the pocket. Using molecular modeling it was determined that a boat cyclohexane ring would replicate the alignment of the two isopropyl groups. Two atoms on one of the leucines create a 1,4-bridge which led to the bicyclo[2.2.2]octane core, which can be further functionalized to mimic the third leucine residue or the helical backbone.[108] These compounds were evaluated using time-resolved fluorescence energy transfer (TRFRET) assays to evaluate their ability to compete with the SRC3 co-activator for the ERα. The results showed that these compounds were modest CBIs with Ki values in the range of 7-40 μM. The best CBI is shown in (**Figure 1.13 (a))**. This is promising because these bicyclo[2.2.2]octanes are actually incomplete helical mimetics. Due to synthetic difficulties of adding an additional amide group, these compounds only mimic one of the leucine residues and the compounds still bind in the low micromolar range.

In 2009 a series of bipolar biphenyl proteomimetic compounds was designed and synthesized by Williams *et.al* as estrogen receptor-α (ERα) coactivator binding inhibitors, based on a bipolar bis-4,4'-oxyphenyl scaffold that addresses both the substitution pattern of the hydrophobic core and the electronic interactions of the charge clamp. Each compound in the series contains a tertiary amine and a carboxylic acid connected by an ether linkage to the biphenyl core. The compound shown in (**Figure 1.13 (b))** exhibits a *K*i of 33 μM and presents as the most promising candidate for follow-up medicinal chemistry in that series. These compounds were subjected to cotransfection reporter gene assays in human endometrial cancer (HEC-1) cells, which express nuclear receptor coactivators but contain no endogenous ERα. The compound in (**Figure 1.13 (b))** shows evidence of inhibitory activity, with an IC_{50} of ~2 μM but only limited inhibitory efficacy.[109]

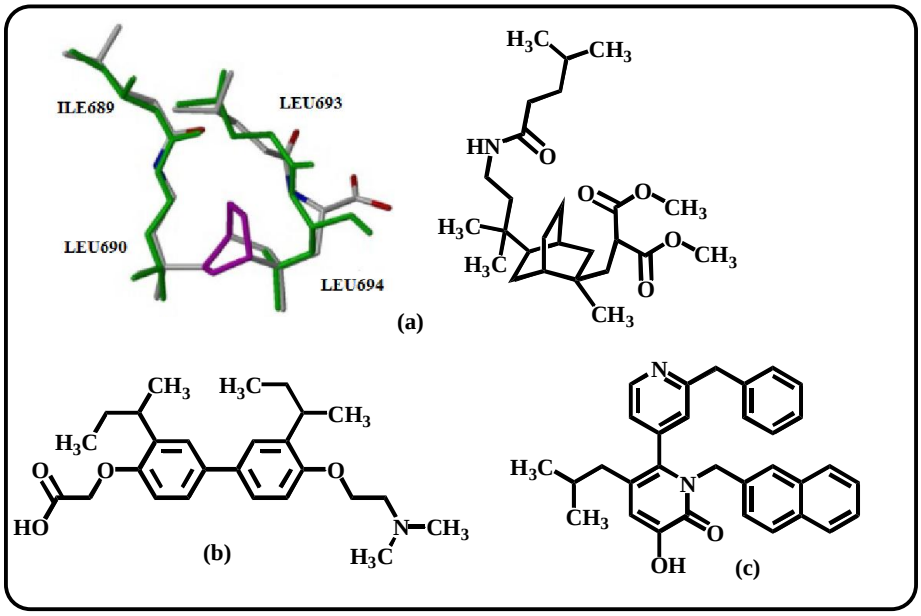


Figure 1.13: **(a)** Cyclohexane connector (purple) creates a bicyclo[2.2.2]-octane CBI core from L690 and L694 of an SRC NR box peptide. **(b)** biphenyl proteomimetic compound with tertiary amine and a carboxylic acid connected to the biphenyl core. **(c)** pyridylpyridone based ERα CBIs.

Hamilton's group has also described a small set of pyridylpyridone based ERα CBIs. These are modified versions of their terphenyl Bcl-xL/Bak inhibitors discussed earlier. This trisubstituted scaffold also has three hydrophobic groups that can project into the CBP. The molecule in (**Figure 1.13 (c))** showed the ability to disrupt ERα/co-activator activity in a fluorescent polarization assay based on rhodamine-labeled peptide D22 which contains a single LXXLL motif with IC_{50} value 4.2 μM.[102]

Another class of compounds that inhibit the ERα/co-activator interaction is based on a pyrimidine core was prepared in 2004. Rodriguez *et al.* had described an approach to developing small molecule coactivator binding inhibitors (CBI) by a de novo, structure-inspired approach, and evaluated a set of candidate CBIs for their activity in blocking the binding of a model nuclear receptor interaction box (NR box) peptide. They took two structure-based approaches where they considered the interactions of the three leucines in the LXXLL motif as an essential requirement for their CBIs based on the concept that the three leucine residues roughly adopt a triangular formation. Basing their search on the crystal structure of LXXLL, they found two sets of molecules (Class I **(a)**

and Class II **(b) (Figure 1.14)**) that could potentially have coactivator binding activity. The first set of CBIs (Class I), based on an outside-in design approach, has various heterocyclic cores (triazenes, pyrimidines, trithianes, cyclohexanes) that mimic the tether sites of the three leucines on the peptide helix. Hydrophobic substituents were then placed in alternate positions around the heterocyclic rings to mimic the leucine residues, and in some cases, polar functionalities were added to interact with the charge clamp residues in the ER LBD. The other set Class II, based on an inside-out approach, has a naphthalene core that mimics the two most deeply buried leucines, with substituents extending outward to mimic other features of the coactivator helical peptide. A fluorescence anisotropy-based coactivator competition assay was developed to measure the specific binding of these CBIs to the groove site on the ER-agonist complex with which coactivators interact by measuring the displacement of a fluorescent labelled octapeptide containing one (NR box) by each CBI; control ligand-binding assays assured that their interaction was not with the ligand binding pocket.[19]

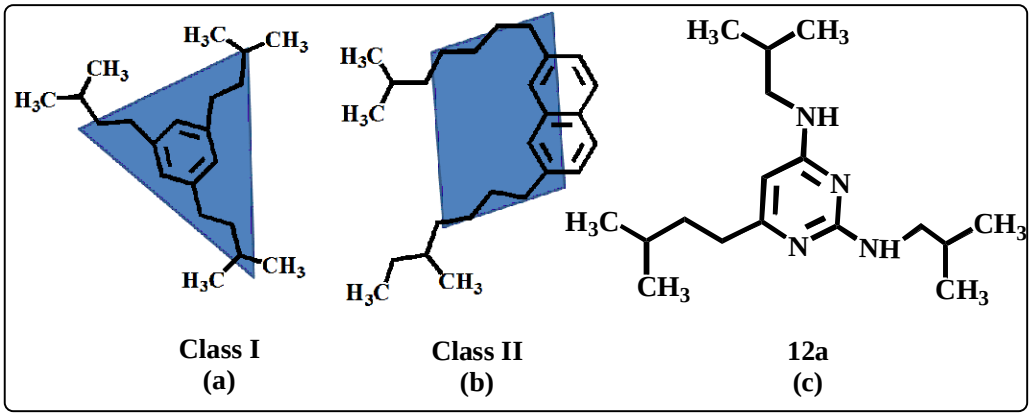


Figure 1.14: Class I **(a)**, Class II **(b)** type molecules using two different approaches; **(c)** the best pyrimidine based ER CBI (12a). Adapted from Rodriguez et al.[19]

After testing triazene, pyrimidine, trithiane, and cyclohexane from Class I**.** Pryimidine cores proved to be the most successful ERα inhibitors with a trialkyl-substituted compound **12a (Figure 1.14 (c))** giving a *K*i of 29 μM, the two alkyl substitutuents of CBI **12a** projecting downward into the deep hydrophobic groove of the receptor, filling the area normally occupied by L690 and L694. The third alkyl substituent sits comfortably on the hydrophobic shelf occupied by I689 in the ERR crystal

structure. The interaction between pyrimidine **12a** and the receptor appears to be entirely hydrophobic in nature, as indicated by the lack of hydrogen bonding or electrostatic interactions in this model **(Figure 1.15)**. To determine whether their best CBI was in fact inhibiting by displacing the coactivator peptide, they measured its relative binding affinity in a radiometric assay with estradiol and ER LBD and obtained a 0.01% RBA of E_2. Hence, as with previous examples, the trialkyl-subsituted pyrimidine was not binding to the LBD but to the ER-coactivator surface as they hoped.[19]

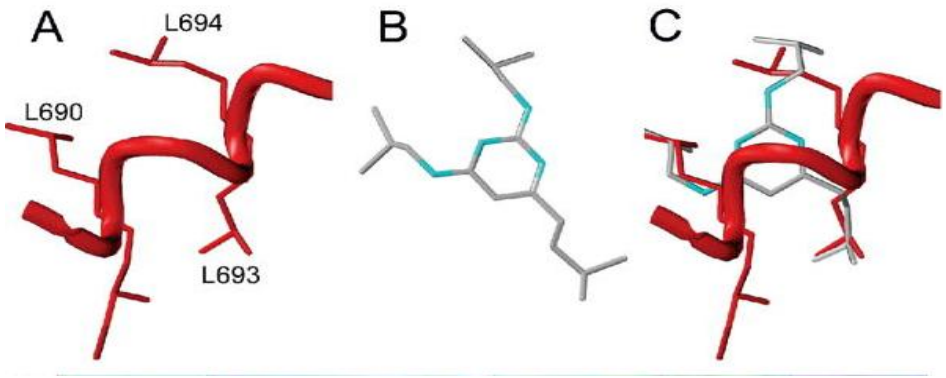


Figure 1.15: pyrimidine CBIs **(12a)** mimicked the leucine side chains with three isobutyl arms. Adapted from Rodriguez et al.[19]

In 2008, Parent *et al*. synthesized a larger library of pyrimidine CBIs based on previous successful of trialkyl-subsituted pyrimidine CBI. A pyrimidine-core library of moderate size, members of which act as α-helix mimics to block ERα/coactivator interaction had been synthesized. Structure-activity relationships had been explored with various C, N, O and S-substituents on the pyrimidine core. Through those studies, a refined pharmacophore model for activity in this pyrimidine series had been obtained. The library of 68 trisubstituted pyrimidines were synthesized and the potency of compounds as inhibitors of the interaction of ERα and ERβ with SRC-3 was characterized using a time-resolved fluorescence resonance energy transfer assay (TR-FRET) and a cell-based E_2-induced reporter gene assay in human cancer cell lines. **(Figure 1. 16)** shows a small subset of the Parent library and their binding affinities. They successfully found a far more potent CBI, **K-11c**, which gave the lowest binding affinity, *K*i of 1.7 μM. Furthermore, the favorable activities of several of these compounds support the feasibility that this coactivator binding inhibition mechanism for

blocking estrogen action might provide a potential alternative approach to endocrine therapy. As before, they identified the type of inhibition of their active pyrimidine CBIs in an *in vitro* competitive radiometric assay with tritium-labeled estradiol as a tracer and full-length purified human ERα and Erβ. The relative binding affinities of their molecules ranged from 0.005 to 0.051% of E_2. Their molecules were indeed inhibiting coactivator association and not through the classical displacement of E_2.[104]

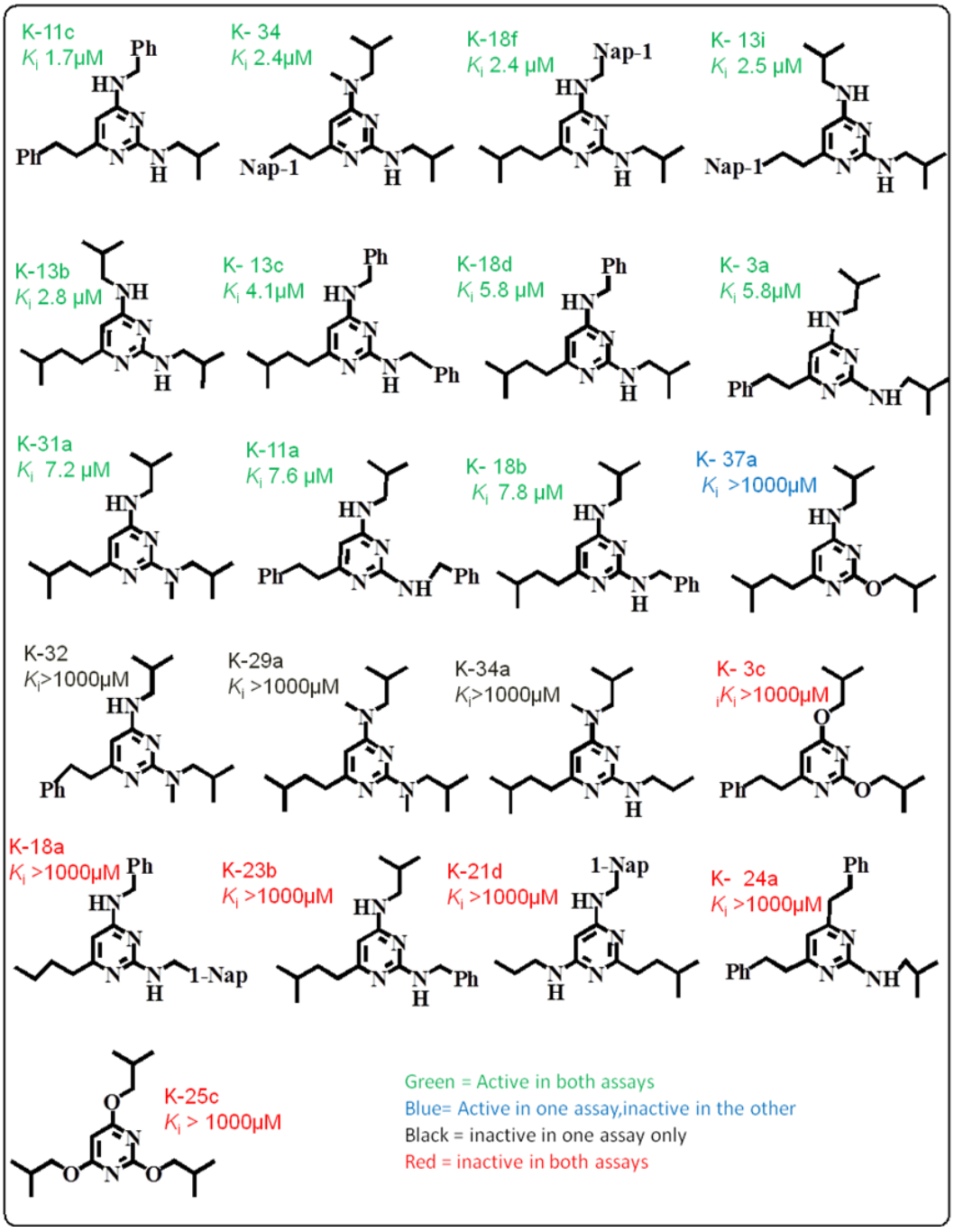


Figure 1.16: A small subset of Parent's pyrimidine CBI library and their biological activities.[104]

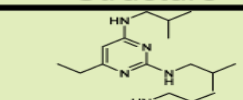
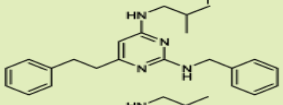
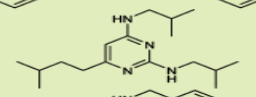
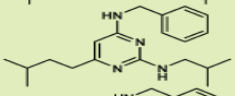
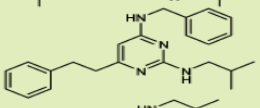
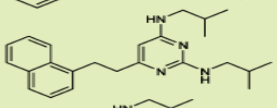
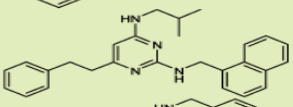
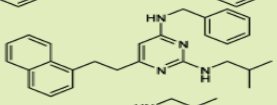
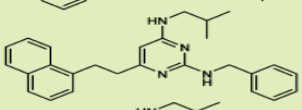
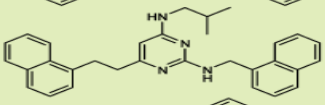
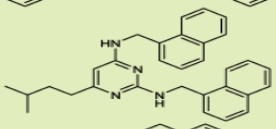
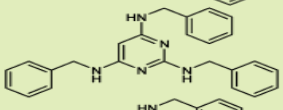
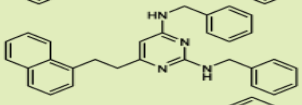
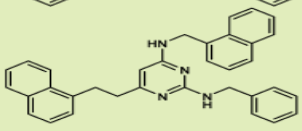
1.5.7. Pyrimidines as a Coactivator Binding Inhibitors (CBIs) of Androgen Receptor (AR)

The androgen receptor (AR) is a member of the nuclear hormone receptor superfamily and plays an integral role in primary and secondary sexual development in males. While abnormalities resulting in an attenuation of the AR response to endogenous hormones (testosterone and its reduced form, 5-dihydrotestosterone or DHT) produce male infertility and feminization, excessive stimulation of the AR can also result in pathologies. The most commonly presented diseases of this type are prostate cancer and the related but benign prostatic hyperplasia. Both of these diseases are responsive to endocrine-based treatments that attempt to suppress tumor/prostate growth either by direct administration of an AR antagonist or by "chemical castration" techniques that result in decreased gonadal production of the endogenous agonist, testosterone.[110] Traditional AR antagonists, such as flutamide or bicalutamide, act by binding to the ligand binding pocket of the receptor, resulting in a conformational change of the ligand binding domain (LBD) such that helix 12 occludes the binding of coactivators that are required to activate transcription. Consequently, this type of inhibition can be considered a type of indirect or allosteric modulation of AR activity, because inhibitor binding in the ligand-binding pocket is disabling a protein_protein interaction at a separate site. While treatment with traditional AR antagonists is initially met with suppression of prostate tumor growth, with time (a few months to years), cellular modifications including AR mutations, upregulation of AR and coactivators, changes in the post-translational modification of AR and accessory proteins, as well as increased androgen production by the suprarenal glands and in the tumors themselves, result in a endocrine-treatment refractory state in which cancer progression occurs despite the presence of an antagonist. As a result, new chemical approaches need to be developed to successfully treat this advanced-stage disease. [111],[112]

Katzenellenbogen *et al.* and others have recently described the evaluation of small CBIs molecules that act as direct protein/ protein disruptors of the interaction between the estrogen receptor (ER) LBD and steroid receptor coactivators (SRCs). Due to the general homology of the external binding

groove of the LBDs of both ER and AR, as shown in crystallographic studies, and the sharing of coactivators containing the LXXLL consensus sequence.[113] Jillian R. Gunther *et .al* hypothesized that compounds containing structural characteristics similar to those that proved effective as ER CBIs would also antagonize the AR/SRC interaction. Additionally, the ability of the AR LBD to bind preferentially to coregulator proteins and peptides containing bulkier aromatic residues suggested that AR-selective CBIs could be formed by simple incorporation of larger side chains on already discovered CBI cores. To test this hypothesis, they designed a compound library based on a 2,4,6-trisubstituted pyrimidine core that had proven effective in earlier ER-CBI work and was designed to mimic the *i, i* + 3, and *i* + 4 arrangement of the three interacting residues of both the ER and AR coactivators **(Figure 1.17**). In addition to the smaller propyl/butyl and isobutyl/ isopentyl groups previously studied, they included larger benzyl/phenethyl and naphthalenemethyl/ naphthethyl moieties in their design to mimic the phenylalanine and tryptophan residues present in the endogenous AR transcriptional system. A structure-based peptidomimetic approach was used to design and synthesize compounds that directly disrupt the androgen receptor/steroid receptor coactivator interaction and function as novel inhibitors of androgen signaling that would remain effective in the treatment of prostate cancer that is resistant to conventional endocrine therapies, based on a pyrimidine-core system. Using fluorescence resonance energy transfer and reporter gene assays, Jillian R. Gunther *et .al* identified members of this library that disrupt the androgen receptor/steroid receptor coactivator interaction selectively, without affecting the estrogen receptor/steroid receptor coactivator interaction. Unlike the activity of traditional androgen receptor antagonists, such as flutamide and bicalutamide, inhibition by these coactivator binding inhibitors is insurmountable by increased concentrations of androgen agonists and maintains effectiveness even on a mutant androgen receptor that is resistant to traditional antagonists. These findings support the feasibility of targeting the coactivator binding groove of the androgen receptor as an alternative approach to treatment-resistant prostate cancer therapy.[114]

Pyrimidine-core coactivator binding inhibitors for ERα and AR

Cmpd #	Structure	IC_{50} (μM) ERα	AR (wt)	AR (T877A)
1		7.9	NB	7.4
2		4.1	2.6	3.5
3		3.6	5.6	7.1
4		3.5	3.0	3.7
5		1.5	1.7	7.5
6		3.5	4.9	3.6
7		>30	1.6	9.4
8		>30	3.3	11.9
9		NB[a]	1.9	3.5
10		NB[a]	1.5	NB
11		NB	6.6	>30
12		NB[a]	3.5	18.9
13		NB[a]	5.6	16.0
14		NB[a]	4.1	>30

[a] K_i measured by TR-FRET. All other values obtained by luciferase reporter gene assay except where noted. Values are averages of duplicate assays generated from 2 or more independent replicates. NB = no binding.

Figure 1.17: A small subset of Jillian R. Gunther *et .al* pyrimidine CBI library and their biological activities.

Some initial docking studies were done on Katzellenbogen *et al.* best CBI using FlexiDock with the SYBYL platform to obtain a clearer picture of the binding mode to ERα. Using the crystal structure of GRIP-1 peptide and overlapping the interactions, they determined that the three alkyl chains of

their CBI were occupying the two deep pockets. However, it was lacking the capping interactions of the peptide with the two polar residues of the charge clamp. In fact, in their study, Lys362 clearly makes an H-bond interaction with the coactivator peptide, but is slightly shifted to the left with their best CBI. It was therefore concluded that no polar or H-bond interactions were involved in the binding modes but simply hydrophobic interactions were made with their CBI and Erα. Lastly, their docking analysis suggested that the ER-coactivator surface is flexible and therefore should be capable of accommodating various sizes of CBIs for future design purposes.[19]

Aims and Objectives of the Study:

The major objective of this study was to explore the possibility to design and prepare a small molecule pyrimidine-based coactivator binding inhibitors (CBIs) that mimic the NR interaction domain containing multiple LXXLL (here L represents leucine and X represents any amino acid) motifs which plays crucial role in the interaction of the NR with steroid receptor coactivator (SRC). CBIs are believed to interrupt the Estrogen Receptor activity by interfering with the interaction of NR-ERα with coregulators (SRC-1, -2, and -3).

Specific Goals of the Study:

1. Design, synthesis, purification, and characterization of series of novel 2,4-disubstitited pyrimidienes.
2. Design, synthesis, purification, and characterization of series 2,4,6-trisubstituted pyrimidine derivatives.
3. Development of appropriate synthetic methods to prepare hetero-trisubstituted pyrimidine-cored derivatives.
4. Assessment of the biological activity of newly 2,4-disubstitited pyrimidines as Acute Myeloid Leukemia (AML) growth inhibitors.
5. Investigate the Structure Activity Relationship (SAR) of the 2,4-disubstitited pyrimidines.

Chapter 2

Experiments and Methods

2. Experiments and Methods

2.1. Synthetic Chemistry*:*

2.1.1. Materials**:**

Chemicals: 2,4,6-trichloropyrimidine **(1)**, 4-fluoroaniline **(2)**, 4-trifluoromethoxyaniline **(3)**, BOC-piperazine **(4),** 1-(5-trifluoromethylpyridin-2-yl)-piperazine **(5),** 1-(3-trifluoromethyl-pyridin-2-yl)-piperazine **(6),** N-phenylpiperazine **(7)**, 2-(1-piperazinyl)pyrimidine **(8),** 1-(4-pyridyl)piperazine **(9)**, 3,4-dimethoxybenzoic **(10),** benzoyl chloride **(11),** ethanolamine **(12),** sodium sulfate (Na_2SO_4), potassium carbonate (K_2CO_3), diisopropylethylamine (DIPEA), dichloromethane (DCM), ethyl acetate (EtOAc), dioxane, hexane, isopropanol, chloroform ($CHCl_3$), *tert*-butanol, triethylamine (TEA), hydrochloric acid (HCl) ethanol (EtOH), hexane, isopropanol, dichloromethan (DCM), chloroform ($CHCl_3$) and hydrochloric acid (HCl) were purchased from ACROS Chemicals Ltd, and were used without further purification.

Silica gel (Silica gel 60 (0.040-0.063 mm)), thin layer chromatography (TLC) (TLC Silica gel 60 F254) sheets were all purchased from Merck Ltd.

Deuterated solvents: D_2O, $CDCl_3$, DMSO-d_6 were purchased from ACROS Chemicals Ltd.

2.1.2. Instrumentation:

2.1.2.1. Nuclear magnetic resonance (^{1}H-, ^{13}C-, NMR):

Data were collected using Varian Unity Inova 300 MHz spectrometer equipped with a 5-mm switchable probe and data were processed using the VNMR software. ^{1}H-NMR chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Spin multiplicities are described as *s* (singl*e*t), *d* (douplet), *t* (triplet), *q* (quartet), and *m* (multiplet).

2.1.2.2. Fourier transform infrared spectroscopy (FTIR):

All infrared spectra were obtained from a KBr matrix (4000–400 cm-1) using a Perkin-Elmer spectrum 100, FT-IR spectrometer.

2.1.2.3. Electrospray ionization mass spectrometry (MS (ESI)):

Was performed using a Thermo Quest Finnigan LCQ-Duo in the positive ion mode. Elution was in a mixture of 49:49:2 water/methanol/acetic acid at a flow rate of 15 μL/minute.

2.1.3. Synthesis of (4-amino-2,6-dichloropyrimidine) derivatives (15-21)

In a 25 ml round bottom flask 2,4,6-trichloropyrimidine **(1)** (1g, 5.45 mmol) was dissolved in dioxane (15 ml). To the mixture the relevant aromatic or aliphatic amine (1.1 mmol) and DIPEA (diisopropylethylamine) (1.2 mmol) were added at room temperature. While stirred, the mixture was heated at reflux for 8–12 hour until completion of the reaction. The reaction progress was followed using TLC. After the reaction completion volatiles were removed under reduced pressure using rotary evaporator. The residue was extracted using ethyl acetate and water (30ml X 3). The organic fractions were pooled and dried using sodium sulfate (Na_2SO_4). Ethyl acetate was removed under reduced pressure to dryness. The crude material was purified by silica gel column chromatography using $CHCl_3$: hexane (40:60; v: v) as eluent to give the 4-amino-2,6-dichloro pyrimidines in 40-70% yield.

2.1.3.1. Synthesis of (2,6-dichloropyrimidin-4-yl)-(4-fluorophenyl)-amine {M1 (15)}:

TLC ($CHCl_3$: hexane (70:30; v: v)), Rf =2.1/5.3=0.39; light brownish powder; yield: 0.9 g (64%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 10.25 (s, 1H), 7.51(d, 2H), 7.18 (d, 2H), 6.68 (s, 1H).

2.1.3.2. Synthesis of 4-(2,6-dichloropyrimidin-4-yl)-piperazine-1-carboxylic acid tert-butyl ester {M2 (16)}:

TLC ($CHCl_3$: hexane (80:20; v: v)), Rf =3.5/6.2=0.51; light gray powder; yield: 0.88 mg (48%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 6.97 (s, 1H), 3.68 (t, 4H), 3.39 (t, 4H), 1.41 (s, 9H).

2.1.3.3. Synthesis of (2,6-dichloropyrimidin-4-yl)-(4-trifluoromethoxyphenyl)-amine {M3 (17)}

TLC ($CHCl_3$: hexane (80:20; v: v)), Rf =1.8/ 6.1= 0.29; white powder; yield: 1.18 g (67%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 10.41(s, 1H), 7.68(d, 2H), 7.40(d, 2H), 6.79 (s, 1H); FT-IR

(KBr) (cm^{-1}): 3311.3(N-H) stretching, 3231.5,3178 Ar-H (C-H stretch), 1568.27 & 1508.3 (C=C) aromatic & (C=N) stretching, 838.56 & 819.89 Ar-H ($H_2C=CH_2$ bending), 967.28 (C-Cl stretch),704.67(C-F stretch).

The compound **(4,6-dichloropyrimidin-2-yl)-(4-trifluoromethoxyphenyl)-amine (23)** was collected from the same column chromatography purification step as a minor product.

2.1.3.4. (4,6-dichloropyrimidin-2-yl)-(4-trifluoromethoxyphenyl)-amine {M9 (23)}:

TLC ($CHCl_3$: hexane (80:20; v: v)), Rf =3.2/ 5.2= 0.61; Off-white powder; yield: 0.3g (17%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 10.58(s, 1H), 7.73(d, 2H), 7.34(d, 2H), 7.21 (s, 1H).

2.1.3.5. Synthesis of 2,4-dichloro-6-[4-(5-trifluoromethylpyridin-2-yl)-piperazin-1-yl]pyrimidine {M4 (18)}

TLC (DCM: hexane (40:60; v: v)), Rf =1.5/6= 0.25; white powder; yield: 1g (49%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 8.4(s, 1H), 7.79(d, 1H), 7.40(d, 1H), 6.95 (s, 1H), 3.75 (t, 8H).

2.1.3.6. Synthesis of 2,4-dichloro-6-[4-(3-trifluoromethylpyridin-2-yl)-piperazin-1-yl]pyrimidine {M5 (19)}

TLC ($CHCl_3$: hexane (80:20; v: v)), Rf = 1.33/ 6= 0.22; white powder; yield: 0.65g (51%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 8.53(d, 1H), 8.09(d,1H), 7.21 (s,1H), 7.06 (t,1H), 3.77(t,4H), 3.29 (t,4H).

2.1.3.7. Synthesis of 2,4-dichloro-6-(4-phenylpiperazin-1-yl)-pyrimidine {M6 (20)}

TLC (DCM), Rf = 2/6= 0.3; white powder; yield: 0.75g (44%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 7.23(d, 2H), 7.09 (s, 1H), 6.96 (d, 2H), 6.81(s, 1H), 3.79(t, 4H), 3.21(t, 4H).

2.1.3.8. Synthesis of 2,4-dichloro-6-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidine {M7 (21)}.

TLC (DCM: EtOAc (80: 20; v: v)), Rf= 3/5.3= 0.56; white powder; yield: 0.68g (41%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 8.39 (d, 2H), 7.06 (s, 1H), 6.67(t, 1H), 3.78 (t, 4H), 3.32(t, 4H).

2.1.4. Synthesis of symmetric analogues of 2,4-diamino-6-chloropyrimidine (MY1 (24), MY2 (25), MY13 (36))

In a 25 ml round bottom flask, 2,4,6-trichloropyrimidine **(1)** (1g, 5.45 mmol) was dissolved in (15 ml) solvent (isopropanol or dioxane). The mixture was then treated with aliphatic or aromatic amine (2.0 mmol) in the presence of base (5.0 mmol) at room temperature. While stirred, the mixture was heated at reflux for 15-22 hour until completion of the reaction. The reaction progress was followed using TLC. After the reaction completion volatiles were removed under reduced pressure using rotary evaporator. The residue was extracted using ethyl acetate and water (30ml X 3). The organic fractions were pooled and dried using sodium sulfate (Na_2SO_4). Ethyl acetate was removed under reduced pressure to dryness. The crude material was purified by silica gel column chromatography using $CHCl_3$: hexane (80: 20; v: v) as eluents to give 2,4-diamino-6-chloropyrimidine.

2.1.4.1. Synthesis of 6-chloro-N2,N4-bis(4-(trifluoromethoxy)phenyl)pyrimidine-2,4-diamine {MY1 (24)}

TLC (DCM: EtOAc (90:10; v: v)); Rf = 2.25/4.3= 0.52; white powder; yield: 0.7g (28%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 9.78(s, 2H); 7.68(d, 4H); 7.26(dd, 4H); 6.22 (s, 1H); FT-IR (KBr) (cm^{-1}): 3248.48 (N-H) stretching, 3012.5 Ar-H (C-H stretch), 2965 CH2 (C-H stretch), 1574.37,1506.9 (C=C) aromatic & (C=N) stretching, 747.16 & 696.83 Ar-H (H2C=CH2 bending),870.38(C-Cl) stretch, 800.8(C-F) stretch.

2.1.4.2. Synthesis of 4,4'-(6-chloropyrimidine-2,4-diyl)bis(1-(5-(trifluoromethyl)pyridin-2-yl) piperazine) {MY2 (25)}

TLC (DCM: EtOAc (90: 10; v: v)); Rf = 2/5.6= 0.35; white powder; Yield: 2.4g (77%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 8.41(s, 2H); 7.81(d, 2H); 9.96 (d, 2H); 6.23 (s, 1H); 3.72 (t,16H); FT-IR (KBr) (cm^{-1}): 2984.9 Ar-H (C-H stretch), 2942.9 CH2 (C-H stretch), 1474.37,1444.02 (C=C) aromatic & (C=N) stretching, 814.41,779.91, 761.26 Ar-H ($H_2C=CH_2$ bending), 858.17(C-Cl) stretch, 728.28 (C-F) stretch.

2.1.4.3. Synthesis of di-tert-butyl 4,4'-(6-chloropyrimidine-2,4-diyl)bis(piperazine-1-carboxylate) MY13 (36)

TLC (DCM: EtOAc (80: 20; v: v)); Rf=3.9 /5.5=0.7; light brown powder; Yield: 1g (53%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 6.18 (s, 1H); 3.61 (t, 8H); 3.32 (t, 8H); 1.40 (s, 18H)**.**

2.1.5. Synthesis of 2,4-diamino-6-chloropyrimidine derivatives: (26, 27, 28, 29, 30, 31, 32, 33, 34, 35)

In a 25 ml round bottom flask, 4-amino-2,6-dichloropyrimidine (1.0 mmol) prepared from the previous procedure was dissolved in (10 ml) solvent (butanol or dioxane). The mixture was then treated with aliphatic or aromatic amine (2.0 mmol) in the presence of DIEPA (5.0 mmol) at room temperature. While stirred, the mixture was heated at reflux for 15-72 hour until completion of the reaction. The reaction progress was followed using TLC. After the reaction completion volatiles were removed under reduced pressure using rotary evaporator. The residue was extracted using ethyl acetate and water (30ml X 3), The organic fractions were pooled and dried using sodium sulfate (Na_2SO_4). Ethyl acetate was removed under reduced pressure to dryness. The crude material was purified by silica gel column chromatography using DCM: hexane (80: 20; v: v) as eluent to give 2,4-diamino-6-chloropyrimidine in 44-85% yield.

2.1.5.1. Synthesis of {6-chloro-2-[4-(5-trifluoromethylpyridin-2-yl)-piperazin-1-yl]-pyrimidin-4-yl}-(4-trifluoromethoxyphenyl)-amine {MY3 (26)}

TLC ($CHCl_3$); Rf =1.5/5.3=0.28; Yield: 0.21g (44%); off-white powder; ^{1}H-NMR 300 MHz ($CDCl_3$) δ (ppm): 9.73(s, 1 N-H); 8.44(s, 1H); 7.84(d, 1H); 7.74 (d, 2H); 7.36(d, 2H). 6.99 (d, 1H), 6.10 (s, 1H), 3.79 (t, 8H); ^{13}C{1H}-NMR (DMSO, δ ppm), 160.97, 160.45, 158.75, 139.31, 122.10, 121.29, 106.73, 94.97, 44.17, 43.57, 40.75, 40.48, 40.20, 39.92, 39.64, 39.37, 39.09. FT-IR (KBr) (cm^{-1}): 3366.6 (N-H) stretching, 2854.1 Ar-H (C-H stretch), 1620.56, 1567(C=C) aromatic & (C=N) stretching, 1498.3 (C-H) bending, 842.7 & 789.62 Ar-H (H2C=CH2 bending), 810.17(C-Cl)

stretch, 708.02 (C-F) stretch, HPLC R_t 8.189 minute; ESI-MS m/z calculated for $C_{21}H_{17}ClF_6N_6O$ 518.84, found 519.04 $(M+H)^+$.

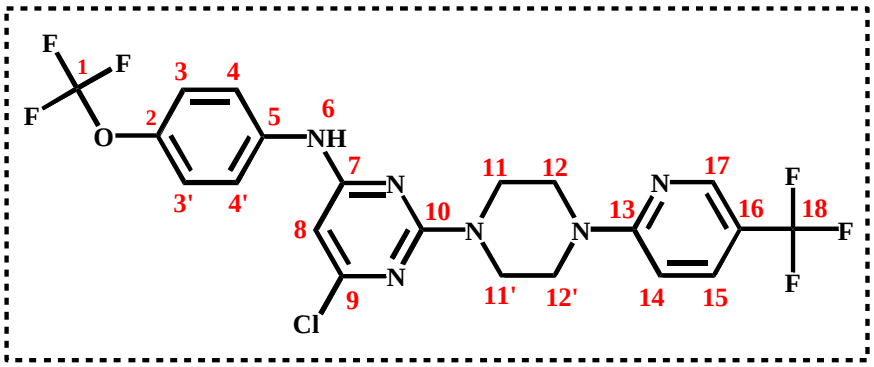


Figure 2.1: Chemical structure of **{MY3 (26)}**

Table 2.1: NMR data of compound **26**

Position	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)
1	-	44.17 (C)
2	-	122.10 (C)
3, 3'	7.74 (d,2H)	40.20 (CH)
4, 4'	7.36 (d,2H)	40.48 (CH)
5	-	106.73 (C)
6	9.73 (s,1H)	-
7	-	160.97 (C)
8	6.10 (s,1H)	39.64 (CH)
9	-	158.75 (C)
10	-	160.45 (C)
11, 11'	3.79 (d,8H)	39.09(CH_2)
12, 12'	3.79 (d,8H)	39.37(CH_2)
13	-	139.31(C)
14	6.99 (d,1H)	39.92 (CH)
15	7.84 (d,1H)	94.97 (CH)
16	-	43.57(C)
17	8.44 (s,1H)	121.29 (CH)
18	-	40.75(C)

2.1.5.2. Synthesis of [6-chloro-2-(4-phenylpiperazin-1-yl)-pyrimidin-4-yl]-(4 trifluoromethoxy phenyl)-amine {MY4 (27)}

TLC (DCM: EtOAc (90: 10; v: v); Rf =2.2/5.2=0.4; dark brown oily compound; Yield: 0.5g (72%); 1H-NMR 300 MHz (DMSO) δ (ppm): 9.64 (s, 1H); 7.65 (d, 2H); 7.27 (d, 2H), 7.16 (d, 2H); 6.93 (d, 2H); 6.73 (t,1H); 6.02 (s, 1H); 3.77(t,4H); 3.23(t, 4H).

2.1.5.3. Synthesis of [6-chloro-2-(4-pyridin-4-yl-piperazin-1-yl)-pyrimidin-4-yl]-(4-trifluoro methoxyphenyl)-amine {MY5 (28)}

TLC (DCM: EtOAc (90: 10; v: v)), Rf=2.4/5.7=0.4; yellow powder; Yield: 0.51g (85%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 9.68 (s, 1H); 8.15 (d, 2H); 7.69 (d, 2H); 7.30 (d, 2H); 6.84 (d, 2H); 6.05 (s, 1H); 3.78 (t, 2H); 3.45(t, 2H). FT-IR (KBr) (cm^{-1}):3092.95 (N-H) stretching, 2964.11 Ar-H (C-H) stretch, 1566.76, 1501.7(C=C) aromatic & (C=N) stretching, 1349.77(C-H) bending, 951.37(C-Cl) stretch, 799.7 Ar-H ($H_2C=CH_2$) bending, 718.42(C-F) stretch.

2.1.5.4. Synthesis of {6-chloro-2-[4-(3-trifluoromethylpyridin-2-yl)-piperazin-1-yl]-pyrimidin-4-yl}-(4-trifluoromethoxyphenyl)-amine {MY6 (29)}

TLC (DCM), Rf =2.5/5.3=0.45 ; off-white powder; Yield: 0.57g (71%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 9.67 (s, 1H); 8.53 (d, 1H); 8.08 (d,1H); 7.70 (d, 2H); 7.29 (d, 2H); 7.11 (t,1H); 6.07(s,1H); 3.8 (t,4H); 3.28 (t, 4H). FT-IR (KBr) (cm^{-1}): 3275.21(N-H) stretching, 2983.6, 2902.7, 2845.08 Ar-H (C-H stretch), 1567.46, 1505.63(C=C) aromatic & (C=N) stretching, 1442.68(C-H) bending, 860.8 (C-Cl) stretch, 795.46, 781.41 Ar-H ($H_2C=CH_2$) bending, 739.86(C-F) stretch.

2.1.5.5. Synthesis of [6-chloro-2-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidin-4-yl]-(4 trifluoro methoxyphenyl)-amine {MY7 (30)}

TLC (DCM: EtOAc (80: 20; v: v); Rf = 3.4/4.8= 0.7; Yield: 0.51 g (72%); off-white powder; FT-IR (KBr) (cm^{-1}): 3271.24(N-H) stretching, 2993.5, 2898.85 Ar-H (C-H stretch), 1473.28, 1440.35 (C=C) aromatic & (C=N) stretching, 1392.11, 1355.74 (C-H) bending, 854.51(C-Cl) stretch,

982.72, 809.81, 787.38 Ar-H ($H_2C=CH_2$) bending. ESI-MS m/z calculated for $C_{19}H_{17}ClF_3N_7O$ 451.83, found 452.07 $(M+H)^+$.

2.1.5.6. Synthesis of 4-{6-chloro-2-[4-(5-trifluoromethylpyridin-2-yl)-piperazin-1-yl]pyrimidin-4-yl}-piperazine-1-carboxylic acid tert-butyl ester {MY8 (31)}

TLC (DCM: EtOAc (80: 20; v: v); Rf =3.2/5.5=0.58**,** Yield: 0.35 g (74%); yellowish oil ; ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 8.45 (s, 1H); 7.84 (d, 1H); 6.98 (d,1H); 6.23 (s, 1H); 3.73 (t, 8H); 3.59 (t,4H); 2.07(s,4H); 1.42 (s, 9H).

2.1.5.7. Synthesis of {4-chloro-6-[4-(5-trifluoromethylpyridin-2-yl)-piperazin-1-yl]-pyrimidin-2-yl}-(4-trifluoromethoxyphenyl)-amine {MY9 (32)}

TLC (DCM: EtOAc (90: 10; v: v); Rf =2.3/5.6=0.41; off-white powder; Yield: 0.4g (72%); ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 9.76(s,1H), 8.45 (s, 1H); 7.82 (d, 2H); 7.62 (d,1H); 7.33 (d, 2H); 7.05 (d,1H); 6.47 (s, 1H); 3.69 (t, 8H).

2.1.5.8. Synthesis of 6-chloro-N4-(4-fluorophenyl)-N2-(4-trifluoromethoxyphenyl)pyrimidine-2,4-diamine {MY10 (33)}

In a 25 ml round bottom flask (1g, 3.1 mmol) of 4,6-dichloropyrimidin-2-yl)-(4-trifluoromethoxyphenyl)-amine **{M9 (23)**} was dissolved in 10 ml dioxane. To the reaction (0.38g, 3.4 mmol) of 4-fluoroaniline **(2)** and conentrated HCl were added. The mixture then was stirred at reflux for 145 hours until the completion of the reaction. The reaction progress was followed by TLC (DCM). After the reaction completion volatiles were removed under reduced pressure using rotary evaporator. The residue was extracted using ethyl acetate and water (30ml X 3). The organic fractions were pooled and dried using sodium sulfate (Na_2SO_4). Ethyl acetate was removed under reduced pressure to dryness. The crude material was purified by silica gel column chromatography using DCM: hexane (90: 10; v: v) as eluent to afford the compound **(33).**

TLC (DCM: EtOAc (90: 10; v: v); Rf=2.3/5.6=0.41**,** Yield: 0.7g (57%) off-white powder; 1H-NMR 300 MHz (DMSO) δ (ppm): 7.94(d,4H),7.60 (t,2H), 7.45(d,4H), 6.21(s, 1H), 3.66(t, 16H). FT-

IR (KBr) (cm^{-1}): 3242.62 (N-H) stretching, 3088.02 Ar-H (C-H stretch), 1577.55, 1505 (C=C) aromatic & (C=N) stretching, 1421.93, 1392.11(C-H) bending, 853.51(C-Cl) stretch, 802.86, 785.44 Ar-H ($H_2C=CH_2$) bending, 733.58(C-F) stretch.

2.1.5.9. Synthesis of (6-chloro-2-piperazin-1-yl-pyrimidin-4-yl)-(4-fluorophenyl)-amine {MY11 (34)}

In a 25 ml round bottom flask (0.8g , 3.1 mmol) of (2,6-dichloropyrimidin-4-yl)-(4-fluorophenyl)-amine {M1 **(15)**} were dissolved in 15 ml dioxane and then treated with (0.64g, 3.45 mmol) of BOC-piperazine (**4)** in the presence of (2 ml , 12.6 mmol) of DIPEA. The mixture then was stirred at reflux at 100 °C for 110 hours until the completion of the reaction. The reaction progress was followed by TLC ($CHCl_3$). After the reaction completion volatiles were removed under reduced pressure using rotary evaporator. The residue was extracted using ethyl acetate and water (30ml X 3). The organic fractions were pooled and dried using sodium sulfate (Na_2SO_4). Ethyl acetate was removed under reduced pressure to dryness. The crude material was purified by silica gel column chromatography using DCM: hexane (70: 30; v: v) as eluent to afford the compound **(34).**

TLC (DCM: EtOAc (90:10; v: v)), Rf=2.8/5.1=0.5; white powder; yield: 0.65g (51%) ; ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 9.53 (s, 1H); 7.56 (d, 2H); 7.18 (d,2H); 6.02 (s, 1H); 3.65 (t, 4H); 3.37 (t,4H); 1. 42 (s, 9H).

2.1.5.10. Synthesis of 4-[4-chloro-6-(4-fluorophenylamino)-pyrimidin-2-yl]-piperazin-1-yl}-phenylmethanone {MY12(35)}

In a 25 ml round bottom flask 0.5g of (6-chloro-2-piperazin-1-yl-pyrimidin-4-yl)-(4-fluorophenyl)-amine {MY11 **(34)}** was dissolved in (10 ml) EtOH and acidified by 0.3 ml concentrated HCl and stirred for 6 hours. Then the mixture was allowed to cool in ice-bath overnight, and the precipitate was filtered off. The precipitate was washed with additional 10 ml ethanol and filtrates were collected and evaporated to dryness to give a 0.45g white precipitate of corresponding (6-chloro-2-piperazin-1-yl-pyrimidin-4-yl)-(4-fluorophenyl)-amine hydrochloride salt. (0.35g, 1.13mmol) of the

latest salt was then dissolved in 10 ml pyridine and then treated with (0.77g, 5.5 mmol) of benzoyl chloride **(11)** in the presence of (785µl, 5.65 mmol) DIPEA. The mixture then was stirred at reflux for 48 hours until the completion of the reaction. The reaction progress was followed by TLC ($CHCl_3$). When the reaction was completed, pyridine was removed by rotary evaporator and the residue was extracted using ethylacetate and water (30ml X 3), traces of water are removed by treating the organic phase with a drying agent (Na_2SO_4). Then the solvent was removed under reduced pressure to afford 220 mg light brown powder **{MY12 (35)}.**

TLC (DCM: EtOAc (90: 10; v: v)); Rf= 2.3/5.1= 0.45; yield: 0.22g (47%); light brown powder; ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 9.53(s,1H),7.57(d,2H), 7.45(m,5H), 7.15(d,2H), 6.02(s,1H), 3.72(t,8H);^{13}C{1H}-NMR (DMSO-d6, δ ppm), 161.74, 158.60, 136.24, 133.18, 130.18, 129.70, 128.92, 127.49, 122.23, 116.0, 115.71, 94.42, 40.78, 39.59, 39.39, 39.12; FT-IR (KBr) (cm^{-1}): 3290.01 (N-H) stretching, 2989.14 Ar-H (C-H stretch), 1565.07, 1502.17 (C=C) aromatic & (C=N) stretching, 1438.14 (C-H) bending, 855.55(C-Cl) stretch, 831.07, 803.56, Ar-H ($H_2C=CH_2$) bending, 705.73(C-F) stretch. HPLC R_t 8.012 minute; ESI-MS m/z calculated for $C_{21}H_{19}ClFN_5O$ 411.86, found 413.19 $(M+H)^+$.

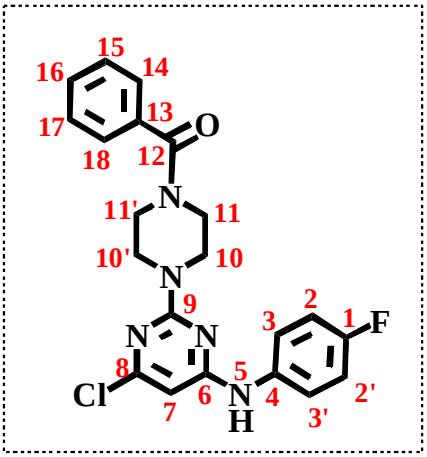


Figure 2.2: Chemical structure of **{MY12 (35)}**

Table 2.2: NMR data of compound **35**

Position	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)
1	-	130.18 (C)
2, 2'	7.57(d, 2H)	39.59 (CH)
3, 3'	7.15 (d,2H)	40.78 (CH)
4	-	129.70 (C)
5	9.53(s,1H)	-
6	-	161.74 (C)
7	6.02 (s,1H)	94.42 (CH)
8	-	133.18 (C)
9	-	158.60 (C)
10,10'	3.72 (t,8H)	39.39 (CH_2)
11,11'	3.72 (t,8H)	39.12 (CH_2)
12	-	136.24 (C)
13	-	128.92 (C)
14	7.45(m,5H)	115.71(CH)
15	7.45(m,5H)	115.17 (CH)
16	7.45(m,5H)	116.0 (CH)
17	7.45(m,5H)	122.23 (CH)
18	7.45(m,5H)	127.49 (CH)

2.1.5.11. Synthesis of 4-chloro-2,6-di-piperazin-1-yl-pyrimidine hydrochloride salt MY14 (37)

In a 25 ml round bottom flask 0.6g of MY13 **(36)** was dissolved in (10 ml) EtOH and acidified by 0.3 ml concentrated HCl and stirred for 6 hours. Then the mixture was allowed to cool in ice-bath overnight, and the precipitate was filtered off. The precipitate was washed with additional 10 ml ethanol and filtrates were collected and evaporated to dryness to give a 0.5g white precipitate of corresponding 4-Chloro-2,6-di-piperazin-1-yl-pyrimidine hydrochloride salt. ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 9.19 (s, 2H); 6.36 (s, 1H); 3.85 (t, 4H); 3.10 (t, 4H).

2.1.5.12. Synthesis of {4-[2-(4-benzoylpiperazin-1-yl)-6-chloropyrimidin-4-yl]-piperazin-1-yl}-phenylmethanone MY15 (38)

In a 25 ml round bottom flask (0.2g, 0.7 mmol) 0f 4-chloro-2,6-di-piperazin-1-yl-pyrimidine hydrochloride salt {MY14 **(37)}** was dissolved in 10 ml pyridine and then treated with (0.22g, 1.6 mmol) of benzoyl chloride **(11)** in the presence of (271µl, 3.5mmol) TEA. The mixture then was stirred at reflux for 22 hours until the completion of the reaction. The reaction progress was followed by TLC ($CHCl_3$: EtOAc (80: 20; v: v)). After the reaction completion, pyridine was removed by rotary evaporator and the residue was extracted using ethyl acetate and water (30ml X 3), The organic fractions were pooled and dried using sodium sulfate (Na_2SO_4). Ethyl acetate was removed under reduced pressure to dryness to afford the product **(38)**.

TLC (CHCl3: EtOAc (80: 20; v: v)); Rf =3.5/5.2= 0.67; yield: 0.3g (87%); light brown powder; ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 7.94(d, 4H), 7.60 (t, 2H), 7.45(d, 4H), 6.21(s, 1H), 3.66(t, 16H).

2.1.6. Synthesis of 2,4,6-trisubstitutedpyrimidine (39, 40, 41, 42, 43, 44, 45, 46, 47)

2.1.6.1. Synthesis of 6-ethoxy-N2,N4-bis(4-(trifluoromethoxy)phenyl)pyrimidine-2,4-diamine {MER1 (39)}

In a 25 ml round bottom flask, sodium metal (1.2 mmol) was dissolved in ethanol (10 ml) and stirred at room temperature for 30-45 minutes. To the mixture (1mmol) of 2,4-diamino-6-chloropyrimidine derivative was then added and refluxed for 90 hours. The reaction progress was followed using TLC (DCM). After the reaction completion, ethanol then was removed by rotary evaporator. The residue was extracted using ethyl acetate and water (30ml X 3). The organic fractions were pooled and dried using sodium sulfate (Na_2SO_4). Ethyl acetate was removed under reduced pressure to dryness to afford the desired 2,4,6-trisubstitutedpyrimidine compounds **(39)**.

TLC (DCM): Rf= 3.6/5=0.75; Yield: 0.15g (75%); brown crystal; ^{1}H-NMR 300 MHz (DMSO) δ (ppm): 9.36 (s, 2H), 7.74 (d, 4H), 7.24 (d, 4H), 5.57 (s, 1H), 4.29 (m, 2H), 1.3 (t, 3H).

2.1.6.2. Synthesis of [6-phenoxy-2-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidin-4-yl]-(4-trifluoromethoxyphenyl)-amine (41), [6-phenoxy-2-(4-phenylpiperazin-1-yl)-pyrimidin-4-yl]-(4-trifluoromethoxyphenyl)-amine (42) and of [6-phenoxy-2-(4-pyridin-4-yl-piperazin-1-yl)-pyrimidin-4-yl]-(4-trifluoromethoxyphenyl)-amine (43).

In a 25 ml round bottom flask, (6 mmol) of sodium was dissolved in dried THF (tetrahydrofurane) (7 ml) in the presence of (3 mmol) phenol, the mixture was stirred at room temperature for 30 minutes. Then (1mmol) of 2,4-diamino-6-chloropyrimidine derivative was added to the mixture and refluxed for 100-120 hours. The reaction progress was followed using TLC (DCM: EtOAc (90:10; v: v)). The reaction was stopped when no progress had noticed, THF was removed by rotary evaporator and the residue was extracted using ethyl acetate and water (30ml X 3), the organic fractions were pooled and dried using sodium sulfate (Na_2SO_4). Ethyl acetate was evaporated to dryness to afford an oily yellow product. A re-crystalization using hexane as solvent was made to purify the compound and to afford the desired compound.

2.1.6.3. [6-phenoxy-2-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidin-4-yl]-(4trifluoromethoxy phenyl)-amine {(MER3(41)}

TLC (DCM: EtOAc (80:20 v: v)); RF=3.2/5.4=0.59; Yield: 0.05g (29%); white oily product..

2.1.6.4. [6-phenoxy-2-(4-phenylpiperazin-1-yl)-pyrimidin-4-yl]-(4-trifluoromethoxyphenyl)-amine {(MER 4 (42)}

TLC (DCM: EtOAc (80:20 v: v)); RF=3.1 /5.4=0.57; Yield: 0.053g (33%); white oily product.

2.1.6.5. [6-phenoxy-2-(4-pyridin-4-yl-piperazin-1-yl)-pyrimidin-4-yl]-(4-trifluoromethoxy phenyl)-amine {(MER5 (43)}

TLC (DCM: EtOAc (90: 10; v: v); RF=3.3/5.4=0.61; white oily product. Yield: 0.22g (21%).yellow oily product.

2.1.6.6. Synthesis of N-(4-fluorophenyl)-N'-(4-trifluoromethoxyphenyl)-2-[4-(3 trifluoromethylpyridin-2-yl)-piperazin-1-yl]-pyrimidine-4,6-diamine (44), N-(4-fluorophenyl)-2-(4-pyrimidin-2-yl-piperazin-1-yl)-N'-(4-trifluoromethoxyphenyl)-pyrimidine-4,6-diamine (45)

In a 25 ml round bottom flask 2,4-diamino-6-chloropyrimidine (1 mmol) was dissolved in dioxane (10 ml). To the mixture 4-fluoroaniline (1.1 mmol) and 200µl of concentrated HCl were added at room temperature. While stirred, the mixture was heated at reflux for 40- 48 hours and followed by TLC (DCM: EtOAc (80:20; v: v)).The reaction was stopped when no progress had noticed. Dioxane was removed by rotary evaporator and the residue was extracted using ethyl acetate and water (30ml X 3). The organic fractions were pooled and dried using sodium sulfate (Na_2SO_4). Ethyl acetate was removed under reduced pressure to dryness. The resulting crude was purified by re-crystallization using DCM as solvent to purify the mixture and to afford the desired compounds.

2.1.6.7. N-(4-fluorophenyl)-N'-(4-trifluoromethoxyphenyl)-2-[4-(3 trifluoromethylpyridin-2-yl)-piperazin-1-yl]-pyrimidine-4,6-diamine {(MER6(44)}

TLC (DCM: EtOAc (80:20; v:v)); RF=3.9/5.6=0.69; brown oil; Yield: 0.45g (20%).

2.1.6.8. N-(4-fluorophenyl)-2-(4-pyrimidin-2-yl-piperazin-1-yl)-N'-(4-trifluoromethoxyphenyl) -pyrimidine-4,6-diamine {(MER7(45)}

TLC (DCM: EtOAc) (80:20; v: v)); Rf= (4.3/6.5)= 0.66; Yield: 0.05g (26%); brown crystal.

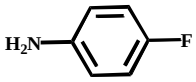
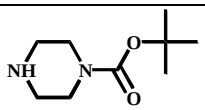
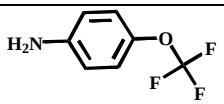
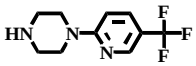
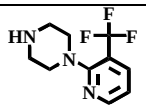
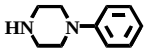
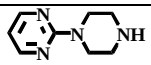
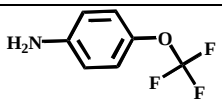
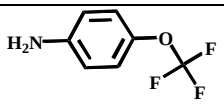
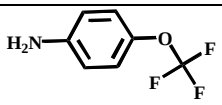
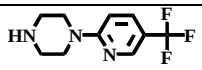
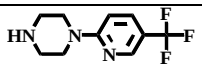
2.1.6.9. Synthesis of {4-[4-(4-benzoylpiperazin-1-yl)-6-(2-hydroxyethylamino)-pyrimidin-2-yl]-piperazin-1-yl}-phenylmethanone {MRE8 (46)}

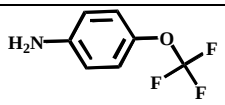
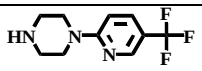
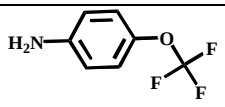
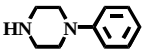
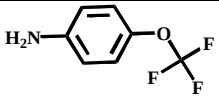
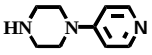
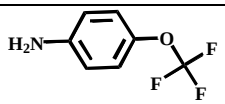
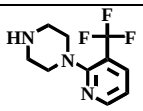
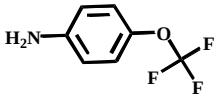
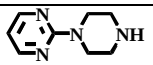
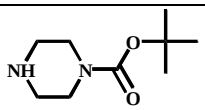
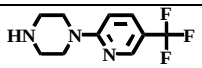
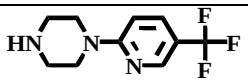
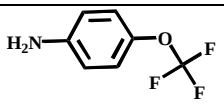
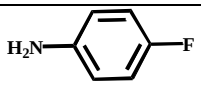
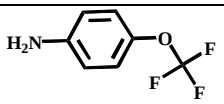
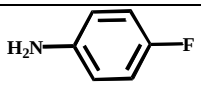
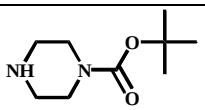
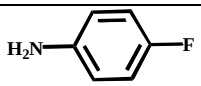
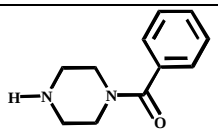
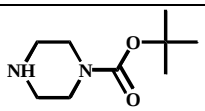
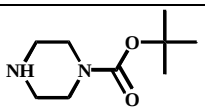
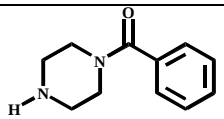
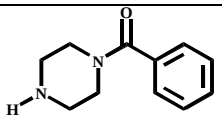
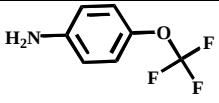
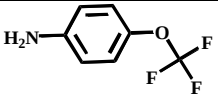
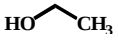
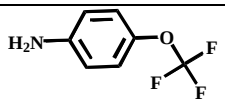
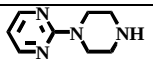
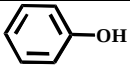
In a 25 ml round bottom flask (0.12g, 0.24 mmol) of {4-[2-(4-benzoyl-piperazin-1-yl)-6-chloro-pyrimidin-4-yl]-piperazin-1-yl}-phenyl-methanone (MY15) **(38)** was dissolved in 10 ml dioxane and then treated with (0.018g, 0.29 mmol) of ethanolamine **(12)** in the presence of (0.1g, 0.72mmol) K_2CO_3. The mixture then was stirred at reflux for 25 hours until the completion of the reaction. The reaction progress was followed by TLC (DCM: EtOAc (80:20; v:v)) .When the reaction was completed, dioxane was removed by rotary evaporator and the residue was extracted using

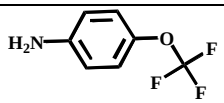
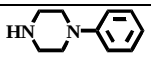
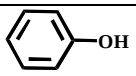
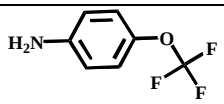
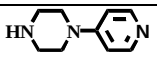
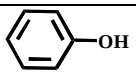
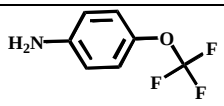
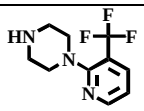
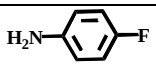
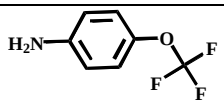
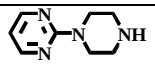
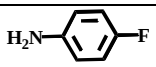
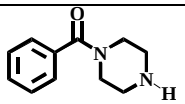
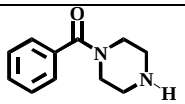
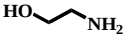
ethylacetate and water (30ml X 3), traces of water are removed by treating the organic phase with a drying agent (Na_2SO_4). Then the solvent was removed under reduced pressure to afford the desired compound.

TLC (DCM: EtOAc (80: 20; v: v)), Rf= 4.2/6.5=; white powder; Yield: 0.04g (33%).

Table 2.3: list of synthesized compounds and their chemical and structural formula.

Com. #	Com. code	**R1**	**R2**	**R3**	Chemical formula	M.wt	yeild
15	M1		Cl	Cl	$C_{10}H_6Cl_2FN_3$	258	64%
16	M2		Cl	Cl	$C_{13}H_{18}Cl_2N_4O_2$	333	48%
17	M3		Cl	Cl	$C_{11}H_6Cl_2F_3N_3O$	324	67%
18	M4		Cl	Cl	$C_{14}H_{12}Cl_2F_3N_5$	378	49%
19	M5		Cl	Cl	$C_{14}H_{12}Cl_2F_3N_5$	378	51%
20	M6		Cl	Cl	$C_{14}H_{14}Cl_2N_4$	309	44%
21	M7		Cl	Cl	$C_{12}H_{12}Cl_2N_6$	311	41%
23	M9	Cl		Cl	$C_{11}H_6Cl_2F_3N_3O$	324	17%
24	MY1			Cl	$C_{18}H_{11}ClF_6N_4O_2$	465	28%
25	MY2			Cl	$C_{24}H_{23}ClF_6N_8$	573	77%

26	MY3			Cl	$C_{21}H_{17}ClF_6N_6O$	519	44%
27	MY4			Cl	$C_{21}H_{19}ClF_3N_5O$	450	72%
28	MY5			Cl	$C_{20}H_{18}ClF_3N_6O$	451	85%
29	MY6			Cl	$C_{21}H_{17}ClF_6N_6O$	519	71%
30	MY7			Cl	$C_{19}H_{17}ClF_3N_7O$	452	72%
31	MY8			Cl	$C_{23}H_{29}ClF_3N_7O_2$	528	74%
32	MY9			Cl	$C_{21}H_{17}ClF_6N_6O$	518	72%
33	MY10			Cl	$C_{17}H_{11}ClF_4N_4O$	399	57%
34	MY11			Cl	$C_{19}H_{23}ClFN_5O_2$	408	51%
35	MY12			Cl	$C_{21}H_{19}ClFN_5O$	412	47%
36	MY13			Cl	$C_{22}H_{35}ClN_6O_4$	483	53%
38	MY15			Cl	$C_{26}H_{27}ClN_6O_2$	491	87%
39	MER1				$C_{20}H_{16}F_6N_4O_3$	474	75%
41	MER3				$C_{25}H_{22}F_3N_7O_2$	509	29%

42	MER4				$C_{27}H_{24}F_3N_5O_2$	507	33%
43	MER5				$C_{26}H_{23}F_3N_6O_2$	508	21%
44	MER6				$C_{27}H_{22}F_7N_7O$	594	20%
45	MER7				$C_{25}H_{22}F_4N_8O$	526	26%
46	MER 8				$C_{28}H_{33}N_7O_3$	515.61	33%

2.2. Comuptational Chemistry

Computer-aided prediction of biological activity of synthesized compounds

The Biological activity is the result of chemical compound's interaction with biological entity. This Biological activity depends on peculiarities of compound (structure and physicochemical properties), biological entity (species, sex, age, etc.), mode of treatment (dose, route, etc.).[115] On the contrary, the biological potential of compound includes all activities, which can be discovered under some specific experimental conditions. The biological potential called the biological activity spectrum. [116] Biological activity spectrum of compound can be predicted on the basis of structure-activity relationships found by the analysis of the known data from the training set. Based on the analysis of large training set consisting of tens of thousands of the known biologically active compounds, computer program PASS (Prediction of Activity Spectra for Substances) provides the means to evaluate any new compound in huge chemical-pharmacological space.[117]

Computer program PASS is the product of ideas originated more than 25 years ago within the framework of the National Registration System of New Chemical Compounds organized in the USSR in 1972.[118] It was V.Avidon who suggested that many classes of biological activity could be predicted on the basis of structural formulae of chemical compounds. Similar approach was under development by V. Golender and A. Rozenblit. [119]

Prediction using the sofware Prediction of Activity Spectra for Substances (PASS)

To predict the bioactivity of our compounds we were assisted by the software Prediction of Activity Spectra for Substances (PASA). PASS predicts simultaneously several hundreds of biological activities (pharmacological main and side effects, mechanisms of action, mutagenicity, carcinogenicity, teratogenicity and embryotoxicity).[120] The biological activity spectrum of a compound presents all compounds' actions despite the difference in essential conditions of its experimental determination. If the difference in species, sex, age, dose, route, etc. is neglected, the biological activity can be identified only qualitatively. Thus, "the biological activity spectrum" is defined as the "intrinsic" property of a compound depending only on its structure and physico-chemical characteristics.[115]

Prediction of this spectrum by PASS is based on SAR analysis of the training set containing more than 35,000 compounds which have more than 500 kinds of biological activity. Therefore, PASS once trained is able to predict simultaneously all biological activities which are included in the training set. To provide the best quality of prediction new information about biologically active compounds is collected permanently from papers and electronic sources and, after the experts' evaluation, is regularly added to the training set. [121]

Chemical descriptors used in PASS analysis, called multilevel neighborhoods of atoms (MNA), are recently considered in detail. They are automatically generated by on the basis of MOL-file of a molecule. The list of MNA descriptors currently consists of more than 35700 different items. The new ones are added to this list being founded in a novel compound refreshing the training set. MNA descriptors are effectively applied in SAR, QSAR and similarity analysis. They can be also used as keys or fingerprints to cluster the libraries of chemical compounds, to select the representative sub-sets from chemical databases, etc.[115]

PASS algorithm was selected by theoretical and empirical comparison of many different mathematical methods to provide high accuracy of prediction and robustness of calculated estimates. It was shown that the mean accuracy of prediction with PASS is about 86% in LOO cross-validation. Using MDDR database to create heterogeneous training and evaluation sets it was recently demonstrated that the predictions are robust despite excluding up to 60% of information.

PASS uses CSV, SD or MOL-files as input and the results of prediction (output) can be obtained as TXT or SD-files. Since the prediction of biological activity spectra for 1,000 compounds in usual PC takes about 1 minute, PASS can be effectively applied to predict biological potential of separate compounds and to analyze large chemical databases.[115]

2.3. Biological studies

The newly synthesized compounds compounds were tested against their biological activity at the lab of Professor Bjørn Tore Gjertsen; Department of Clinical Science. University of Bergen-NORWAY.

Cell line

Acute monocytic leukemia (Molm-13) cell line had been utilized. Molm-13 was obtained from a 20 years old Acute Myeloid Leukemia (AML) patient. This cell line had been stably transfected by sh-RNA (empty vector) and sh-p53 RNA (sh-p53). [122]

***In vitro* Assay**

The viability of the cells was determined using WST-1 assay. The assay is based on the conversion of the tetrazolium salt WST-1 into a colored dye by mitochondrial dehydrogenase enzymes. The soluble salt is released into the media, and within a given time period, the reaction produces a color change which is directly proportional to the amount of mitochondrial dehydrogenase in a given culture as shown in. As a result, the assay actually measures the net metabolic activity of cells

treated by the compound. Theoretically, this is reflective of cell number and can be adopted for use in measuring cell viability at specific concentrations.[123]

Medium

RPMI 1640 Medium was used to perform biological testing of compounds. This medium was originally developed by Moore and his colleagues at Roswell Park Memorial Institute (RPMI) in 1966. RPMI has successfully been used for the cultivation of normal human and neoplastic leukocytes.It has been widely used in fusion protocols and in the growth of hybrid cells.[124]

Chapter 3

Results and Discussion

3. Results and Discussion:

The major challenge facing treatments for tumors is to overcome resistance developed following repeated administration of chemotherapeutics. Controlling the transcription of ER and/or AR-regulated genes by directly inhibiting the interaction of the (ER)/ (AR) with coactivator proteins may provide an alternative mechanism for blocking estrogen or androgen and restrict their activity in breast or prostate cancer offering a solution for the resistance.

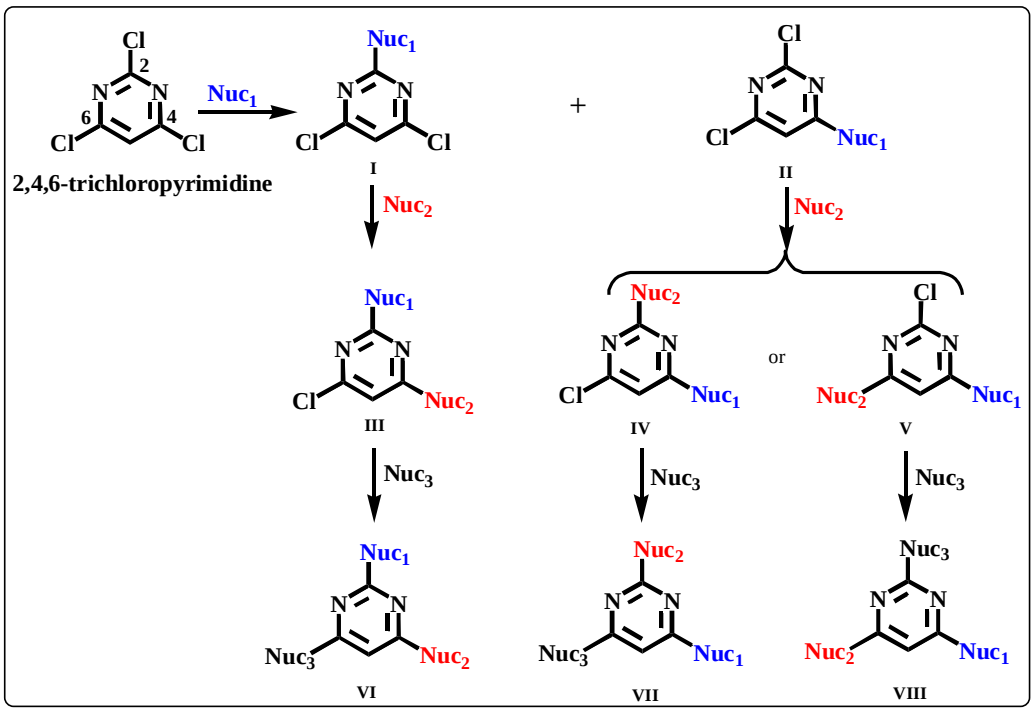
3.1. Chemical synthesis of compounds

Pyrimidine core scaffolds bearing multiple functionality that may be transformed into a widely diverse range of functionalized derivatives by a sequence of efficient and regioselective reactions are becoming increasingly important. Pyrimidines are electron-deficient aromatic systems and, when halogenated, become very useful substrates for a variety of nucleophilic aromatic substitution (S_NAr) processes. Due to the numerous commercial availability of chloro and poly-chloropyrimidines, many synthetic strategies concerned with creating pyrimidine-based libraries from halogenated core scaffolds were reported. In the current study 2,4,6-trichloropyrimidine is used as the core scaffold. However, since regioisomeric products are formed in nucleophilic aromatic substitution stages, separation steps of isomers is required following each step, making adoption of this scaffold less likely. Therefore, there remains a requirement for efficient synthetic methodology that allows the synthesis of regioselective polysubstituted pyrimidines in sequential nucleophilic aromatic substitution. Such synthetic methods might help in meeting the demand for rapid analogue synthesis (RAS) techniques for applications in parallel synthesis.[125]

Reactions of 2,4,6-trichloropyrimidine with amines or other nucleophiles suffers from poor selectivity and diminished regioselectivity, producing mixtures of the 2-substituted and 4-substituted regioisomers **(I, II)** [**Scheme 3.1**] respectively. Moreover, separation and identification of isomers 2-substituted and 4-substituted may be difficult. Purification must be performed to

remove the 2-substituted regioisomer from the mixture before further reactions can be carried out. However, 4-substituted derivatives can be isolated in acceptable yields, further selective functionalization of 4-substituted to form either **IV** or **V** [**Scheme 3.1**] may be similarly complicated.[126]

One problem which in using of 2,4,6-trichloropyrimidine as starting material is related to the reactivity of the three chloride atoms: the formation of the bond between the nucleophilic group and the link group may involve practically any of the three atoms, which is a challenging factor obtaining and purifying the desired product with acceptable yields.



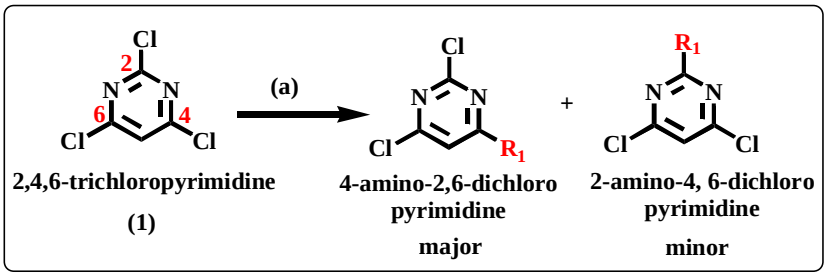
Scheme 3.1: Sequential substitution of the of 2,4,6-trichloropyrimidine by various nucleophiles. Typical methods for preparation of monosubstituted (**I, II**), disubstituted (**III, IV, V**), and trisubstituted pyrimidines (**VI, VII, VIII**). **Nuc**: Nucleophile.

As indicated in **[Scheme 3.1]** three groups of compounds have been synthesized in the course of the current study. The final products classified as 2,4,6-trisubstituted pyrimidines were synthesized in a way to mimic the LXXLL coactivator peptide with a hydrophobic functional groups mimicking the leucine residues in the LXXLL motif, and with a functional groups at the termini to compliment the "charge clamp" of the NR box binding site for the ERα and/or AR.

3.1.1. Step-1: Mono-aminosubstituted pyrimidines (15, 16, 17, 18, 19, 20, 21, 23).

At this stage we aimed at exploring the possibility of introducing different nucleophilic moieties (amines, alkoxides, etc...) as first substituents on the pyrimidine. The reaction was done using reported procedures according to the type of the nucleophile. In case of the first amination a procedure described by Vasudeva Sagi *et al.* was followed.[34] The 4-monoamino-2,6-dichloropyrimidines **(15, 16, 17, 18, 19, 20, 21, 23)** were synthesized using dioxane as solvent instead of ethanol, DIPEA instead of Na_2CO_3 see **[Scheme 3.2]**. In agreement with the other studies, it was noticed that despite a preference toward the 4-position the reaction was not totally regioselective [127],[126]. Thus, mixtures of the 4-amino isomer (major) and 2-amino isomer (minor) were obtained indicating that chloride residing at position-4 is often more labile than the one at position-2. Separation of the two isomers was accomplished using column chromatography prior proceeding to the next step (see **[Scheme 3. 2]** below).

Scheme 3. 2: General strategy for the synthesis of monoaminopyrimidine derivatives



Reagents and conditions: a) Dioxane, DIPEA. **R_1**= aliphatic or aromatic amine, stirring at reflux 100 ˚C for 8-12 hours.

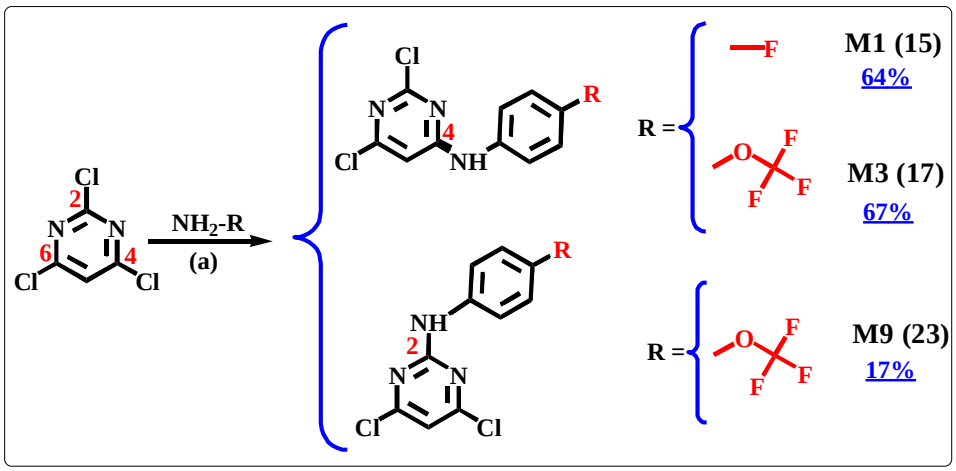
3.1.1.1. Synthesis of (4-amino-2,6-dichloropyrimidine) derivatives (15-21, 23)

The planned compounds were prepared starting from the commercially available 2,4,6 trichloropyrimidine **(1)** that was reacted with different aromatic amines (anilines derivatives) **[Scheme 3.3**] or aliphatic amines (piperazine derivatives) **[Scheme 3.4**] under basic conditions using DIPEA as a base and dioxane as solvent. The nucleophilic displacement of the chloro group by amines took place under elevated temperatures and a mixture of the 2-amino and 4-amino regioisomers was produced. Following evaporation of volatiles and washing with distilled water the

two isomers were purified by column chromatography using $CHCl_3$:hexane (40: 60; v: v). Although these two sets of compounds have been prepared under the same conditions a difference in the percentage of yields was noticed. The 4-anilinopyrimidine derivatives were isolated in good yields that range between 64% for **(15)** to 67% for **(17)** . While the 4-aminopyrimidines with piperazine derivatives had been isolated (41%-51%) yields. Purified intermediates 4-aminopyrimidines **(15-21)** were used in the next steps.

The 2-aminopyrimidine derivative **(23)** which is the isomeric product of **(17)** resulted from reaction of **(1) and (3)** was collected as a white powder in 17% yield and also used in further reactions.

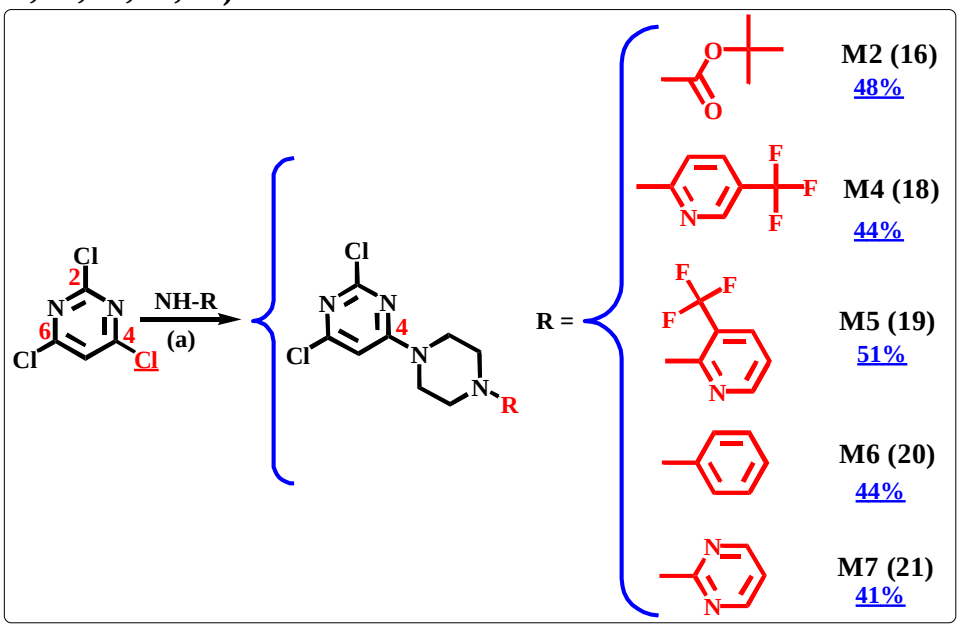
Scheme 3.3: Representative procedure for the synthesis of (4-amino-2,6-dichloropyrimidine) derivatives **(15, 17, 23)**.



Reagents and conditions: a) Dioxane, DIPEA, stirring 8 hours for **15, 17, 23** at 100 ˚C, evaporation, extraction, column chromatography.

Piperazine nucleus is important aliphatic heterocyclic moiety that is found in various compound classes that exhibit wide range of pharmacological activities including antipsychotic, anticonvulsant, antiarrhythmic, antimicrobial, antioxidant, antimalarial, and cytotoxic activities.[128] Different piperazine derivatives were introduced to pyrimidine core such as (BOC-piperazine **(4),** **(5),** 1-(3-trifluoromethyl-pyridin-2-yl)-piperazine **(6),** phenylpiperazine **(7)**, 2-(1-piperazinyl)pyrimidine **(8)**.

Scheme 3.4: Representative procedure for the synthesis of (4-amino-2,6-dichloropyrimidine) derivatives **(16, 18, 19, 20, 21)**.

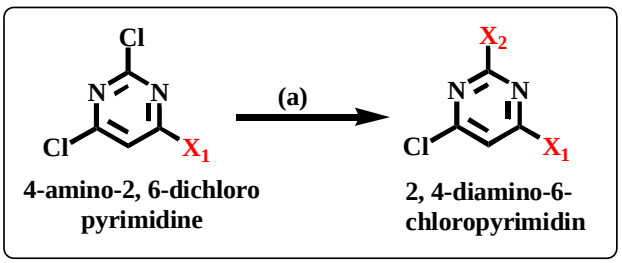


Reagents and conditions: a) Dioxane, DIPEA, stirring 9 hours for **18,** 10 hours for **16,** 12 hours for **19, 20 and 21** at 100 ˚C, evaporation, extraction, column chromatography.

3.1.2. Step-2: Synthesis of 2, 4-diamino-6-chloropyrimidine:

To prepare the 2,4-diamino-6-chloropyrimidines **(26, 27, 28, 29, 30)** the purified 4-amino-2,6-dichloropyrimidine **(17)** was reacted with desired amine. It was noticed that the introduction of the first amine at position-2 induces significant deactivation toward the following amination. Hence the need for elevated reaction temperature, high concentrations of nucleophiles and prolonged times to drive reactions to completion.[129] The procedure described previously by Vasudeva Sagi *et al.* was followed using (2.0 mmol) of the nucleophiles in the presence of DIPEA resulting in displacement of chloro group on position-2 of pyrimidine core[34] (see **[Scheme 3.5]** below).

Scheme 3.5:-General strategy for the synthesis of disubstitutedpyrimidine derivatives.



Reagents and conditions: (a) DIPEA, butanol, reflux at 100 °C, extraction, column chromatography using $CHCl_3$: hexane (80: 20; v: v). X_1, X_2= aliphatic or aromatic amine.

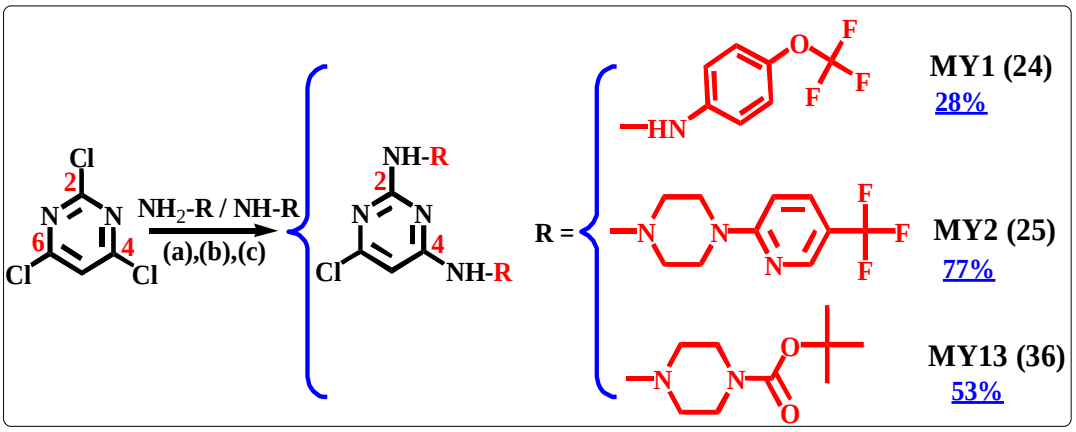
3.1.2.1. Synthesis symmetric of 2,4-diaminopyrimidine analogues (24, 25, 36).

These compounds were synthesized in one step by reacting 1.0 equivalent of **(1)** with 2.0 equivalents of either the aliphatic or aromatic amines. 6-chloro-N2,N4-bis(4-(trifluoromethoxy)phenyl) pyrimidine-2,4-diamine **(24)** was synthesized using 4-trifluromethoxyaniline **(3)** in isopropanol for 22 hours, while for the compound 4,4'-(6-chloropyrimidine-2,4-diyl)bis(1-(5-(trifluoromethyl)pyridin-2-yl)piperazine) **(25)**, **(5)** used with dioxane for 15 hours. (**24** and **25**) were purified by column chromatography using $CHCl_3$: hexane (80: 20; v: v), in 28%, 77% yield respectively. It was noticed that percentage of yield was affected by solvents. Seemingly, the difference of about 20 ˚C degrees in the refluxing temperature of the two solvents could be one of the reasons for the increase in the percentage of yield.

The synthesis of di-*tert*-butyl-4,4'-(6-chloropyrimidine-2,4-diyl)bis(piperazine-1-carboxylate) **(36)** 1.0 equivalent of **(1)** was reacted with 2.0 equivalents of BOC-piperazine in the presence of DIPEA and 0.1g of palladium acetate ($Pd(OAc)_2$ as catalyst (see **[Scheme 3.6])**.

The reaction was over in 4 hours. However, the reaction yield was 53% indicating the effectiveness of $Pd(OAc)_2$ in shortening the reaction time with no improvement in percentage yield.

Scheme 3.6: Representative procedure for the synthesis of 2,4-diamine symmetrical analogues **(24, 25, 36).**



Reagents and conditions: a) TEA, isopropanol. reflux at 100 °C for 22 hours, extraction, column chromatography using $CHCl_3$: hexane (80: 20; v: v). **b)** DIPEA, dioxane; reflux at 100 °C for 15 hours, extraction, column chromatography using $CHCl_3$: hexane (80: 20; v: v). **c)** DIPEA, dioxane;

palladium acetate, reflux at 100 °C for 4 hours, extraction, column chromatography using $CHCl_3$: hexane (80: 20; v: v).

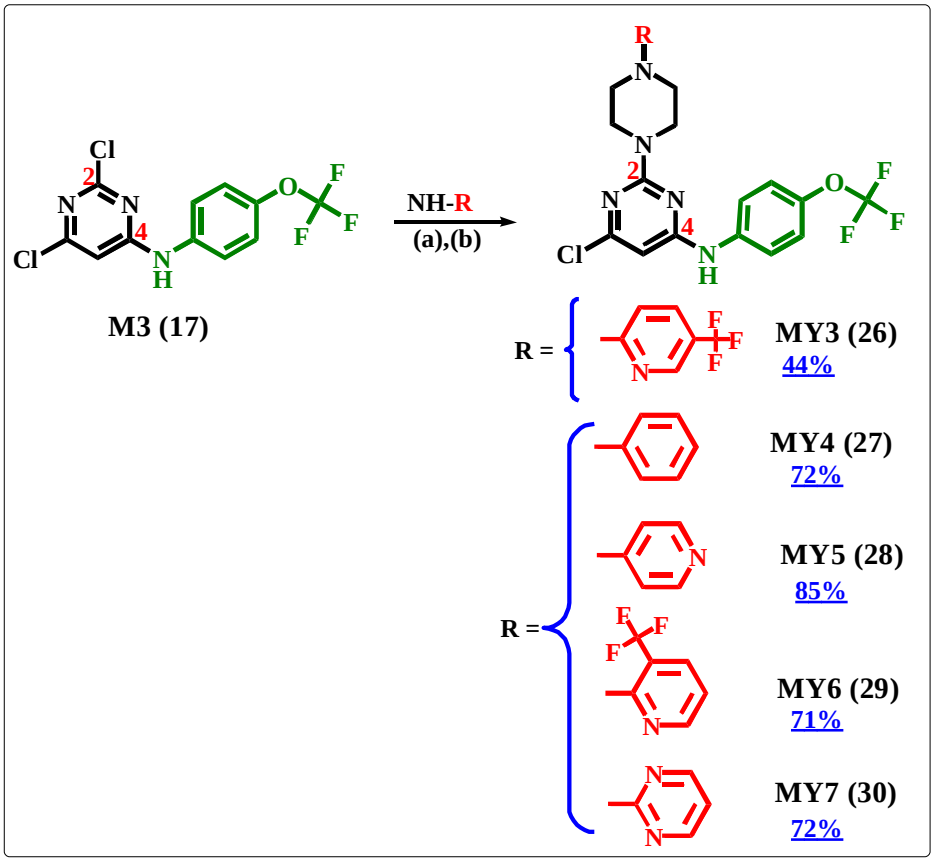
3.1.2.2. Synthesis of 2,4-diamino-6-chloropyrimidine analogues (26-30)

These analogues were generated via nucleophilic substitution at C2-position of pyrimidine core in the presence of various piperazine derivatives in basic conditions under refluxing for about two weeks. (2,6-dichloropyrimidin-4-yl)-(4-trifluoromethoxyphenyl)-amine **(17)** was subjected to further derivatization at C2-position by a different piperazine derivative to generate **(26-30)**. In this series of compounds 4-trifluoromethoxyanilline was retained at C4-position while the substituent at C2-position was modified to give rise to asymmetric 2,4-diaminopyrimidines.

For the synthesis of {6-chloro-2-[4-(5-trifluoromethylpyridin-2-yl)-piperazin-1-yl]-pyrimidin-4-yl}-(4-trifluoromethoxyphenyl)-amine **(26)** similar reaction conditions were used M3 **(17)** with 1 equivalent of **(5)** in the presence of 1.2 equivalent of DIPEA and dioxane. The desired compound was purified by column chromatography using $CHCl_3$: hexane (70: 30; v: v) to afford 210mg off-white powder in 44% yield. The procedure depicted below in [**Scheme 3.7**].

The other compounds **(27-30)** as depicted below in **[Scheme 3.7]** were prepared by using the same precursor **(17)** as a starting material but with different conditions in an attempt to get a higher yield. These reaction were performed by using *tert*-butanol as a solvent instead of dioxane, using 2 equivalents of the piperazine derivatives **(6),** phenylpiperazine **(7)**, **(8),** 1-(4-pyridyl)piperazine **(9)**, and using of 5 equivalents of DIPEA. Purification through column chromatography performed and gave pure compounds with a percentage yield in the range (71%-85%). The improvement in percentage yield may be a result of equivalency increment and of using excess of reagents which pushed the reaction to completion.

Scheme 3.7: General procedure for the synthesis of asymmetric 2,4-diamino-6-chloropyrimidines **(26-30).**

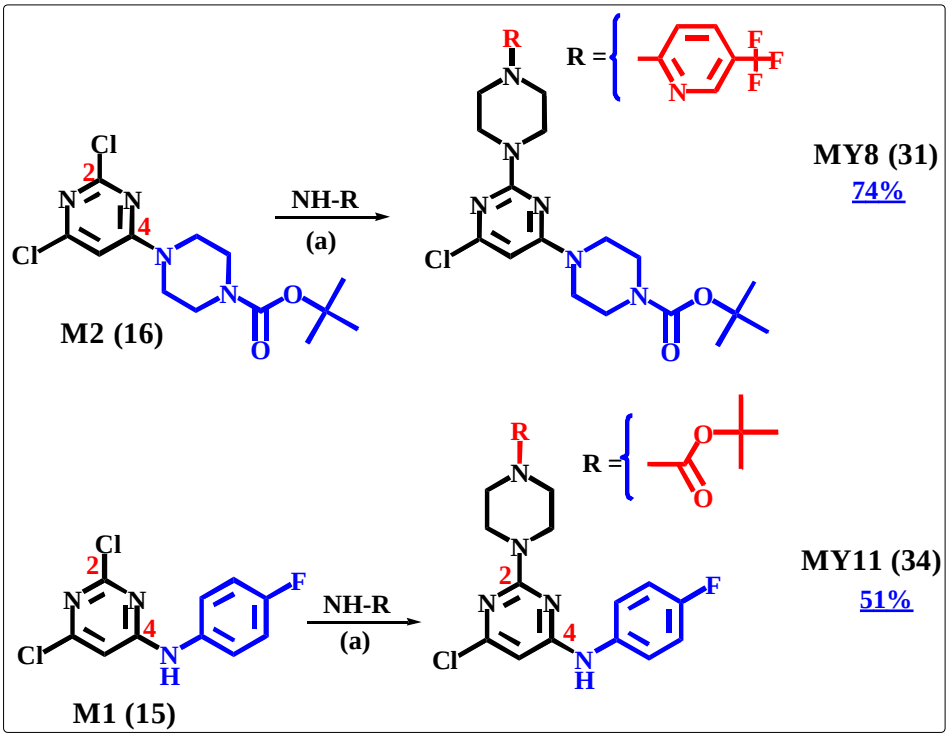


Reagents and conditions: a) Dioxane, DIPEA, reflux at 100 °C for 50 hours, extraction, column chromatography using $CHCl_3$: hexane (70: 30; v: v) **b)** DIPEA, butanol, reflux at 100 °C, for 50 hours for **(27)**, 60 hours for **(28)**, 72 hours for **(29)**, 70 hours for **(30)**.

3.1.2.3. Synthesis of 4-{6-chloro-2-[4-(5-trifluoromethylpyridin-2-yl)-piperazin-1 yl]pyrimidin-4-yl}-piperazine-1-carboxylic acid *tert*-butyl ester {MY8 (31)}, 4-[4-Chloro-6-(4-fluorophenylamino)-pyrimidin-2-yl]-piperazine-1-carboxylic acid *tert*-butyl ester {MY11 (34)}

These two reaction had been performed with dioxane and DIPEA to produce different asymmetrical 2,4-diaminopyrimidine derivatives. The precursor 4-(2,6-dichloropyrimidine-4-yl)-piperazine-1-carboxylic acid *tert*-butyl ester **(16)** treated with **(5)** to give **MY8 (31)**. The precursor (2,6-dichloropyrimidin-4-yl)-(4-fluorophenyl)-amine **(15)** was reacted with BOC-piperazine **(2)** to produce the compound **MY11 (34)**. Extraction and column chromatography using $CHCl_3$: hexane (70: 30; v: v) were performed to purify the compounds. The procedures depicted below in **[Scheme 3.8]**.

Scheme 3.8: Representative procedure for the synthesis of 2,4-diamino-6-chloropyrimidines **(31, 34)**



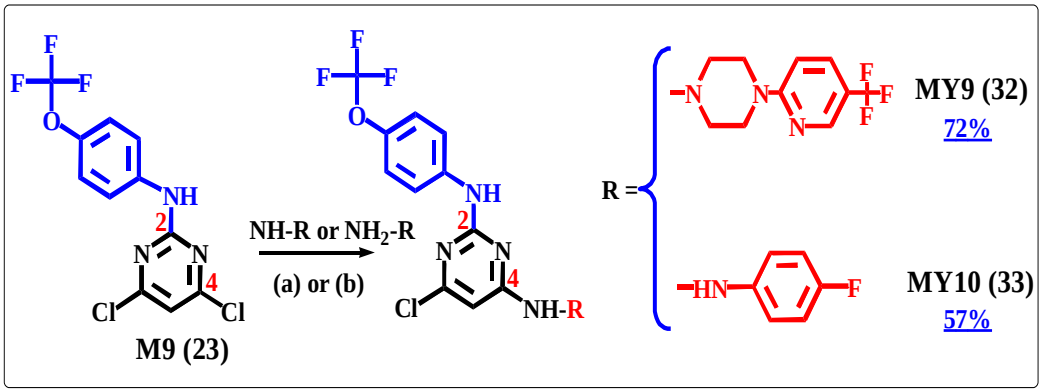
Reagents and conditions: a) Dioxane, DIPEA, reflux 65 hours for **31**, 70 hours for **34.** Extraction and column chromatography using $CHCl_3$: hexane (70: 30; v: v).

3.1.2.4. Synthesis of {4-chloro-6-[4-(5-trifluoromethylpyridin-2-yl)-piperazin-1-yl]-pyrimidin-2-yl}-(4-trifluoromethoxy-phenyl)-amine {MY9 (32)} and 6 -chloro-N4-(4-fluorophenyl)-N2-(4-trifluoromethoxy-phenyl)pyrimidine-2,4-diamine {MY10(33)}.

These two reactions differ from the previous reactions in the isomers that used as a reagent for starting the reaction, all the compounds **(24-31,** and **34)** were produced from a 4-position isomer while the compound **(32)**, **(33)** was produced from a 2-position isomer but lead to the same results of producing different asymmetrical 2,4-diaminopyrimidine derivatives. The compound MY9 **(32)** was produced by treating M9 **(23)** with 1.2 equivalents of **(5)** and DIPEA for 90 hours [**Scheme 3.9**]. The compound MY10 **(33)** was produced by treating M9 **(23)** with 1.2 equivalents of 4-fluoroaniline **(2)** in the presence of 37% HCl.The reactions were stirred at reflux until the reaction completed. The desired compounds were purified by extraction and column chromatography using $CHCl_3$: hexane (80: 20; v: v) and collected as an off- white powders in 72% for **(32)** and 57% for

(33) [**Scheme 3.9**]. Using the acidic conditions for compound MY10 **(33)** had influenced the percentage of yield and the time of reaction.

Scheme 3.9: Representative procedure for the synthesis of 2, 4-diamino-6-chloropyrimidines **(31-33)**

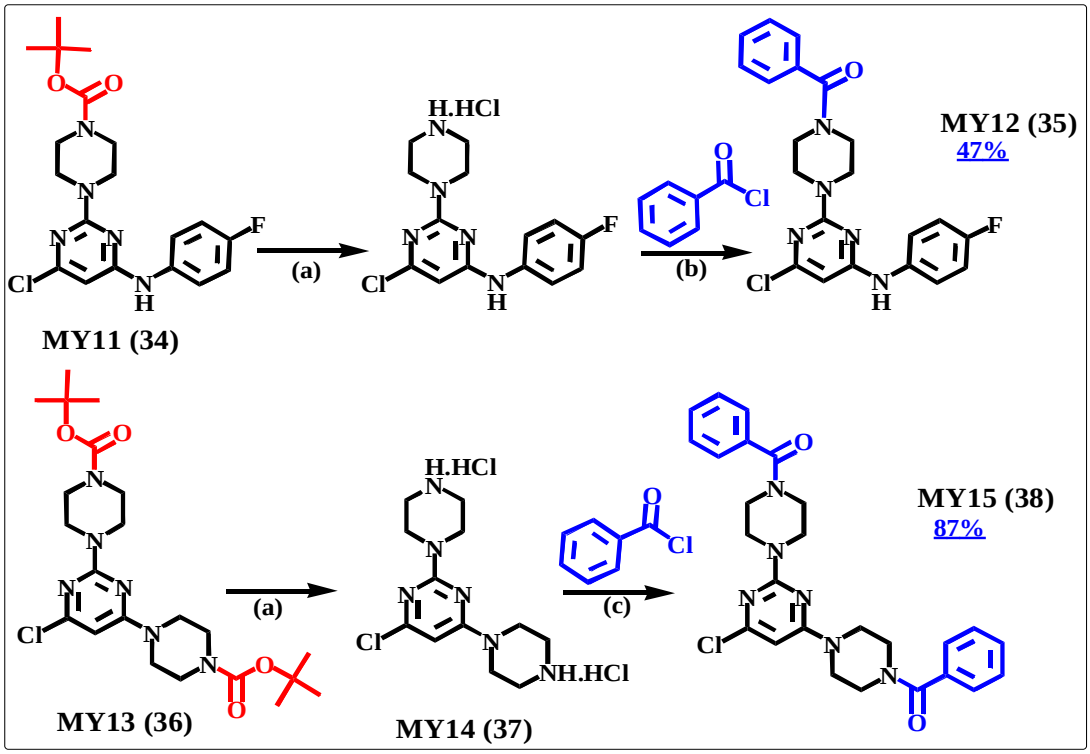


Reagents and conditions: a) dioxane, DIPEA, stirring 65 hours for **31**, 90 hours for **32** at 100 ˚C, evaporation, extraction, column chromatography; or **b)** dioxane, 37% HCl, stirring 140 hours for **33** at 100 ˚C, evaporation, extraction, column chromatography using DCM: hexane (90:10; v:v).

3.1.2.5. Synthesis of 4-[4-chloro-6-(4-fluorophenylamino)-pyrimidin-2-yl]-piperazin-1-yl} phenylmethanone {MY12 (35)}, {4-[2-(4-benzamidylpiperazin-1-yl)-6-chloro-pyrimidin-4-yl]-piperazin-1-yl}-phenyl-methanone MY15 (38)

The first step in preparing these compounds is to cleave the protecting group BOC. The aim of using boc piperazine as a substituent is to prepare new piperazine derivatives that are not commercially available and so to get diversity in the resulted compounds. Compound **(34)** with one protecting group **BOC** at C2-position was treated under acidic conditions to afford the amine free form of piperazinylpyrimidine as white salt **(34),** this salt was then treated with as depicted in **[Scheme 3.10]** under basic conditions with benzoyl chloride to produce **(35)** with benzoylamidylpiperazine substitutent at C2-position as a light brown powder in yield around 47%. **(35)** was characterized by ^{1}H-NMR, FT-IR and ESI-MS spectroscopy. **(38)** Was prepared by cleaving the BOC protecting groups at 2 and 4-position in the symmetrical 2,4-diaminopyrimidine **(36)** under acidic conditions to get MY14 **(37)** as hydrochloride salt**. (37)** was treatted with 2 equivalents of benzoyl chloride **(11)** under basic conditions to form **(38)** with two benzamide piperazine derivatives at 2 and 4- position. The procedures depicted in [**Scheme 3.10**].

Scheme 3.10: Representative procedure for the synthesis of **(35)** and **(38)**:

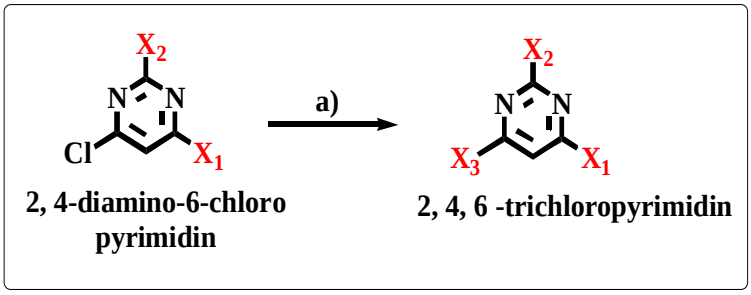


Reagents and conditions: a) EtOH, HCl(aq), stirring 6 hours, cooling, filtration, **b)** Pyridine, DIPEA, reflux 48 hours extraction and evaporation of solvent. **c)** Pyridine, TEA, reflux 22 hours extraction and evaporation of solvent.

3.1.3. Step-3: Synthesis of 2,4,6-trisubstitutedpyrimidines

This is the third and last step in preparing 2,4,6-tisubstituted pyrimidines. This step takes place by attacking the chlorine atom at the 6-position. After the occupation of the 2 and 4 positions of pyrimidine core with an amino groups, the attacking of the third chlorine in 2,4-diamino-6-chloropyrimidine compounds becomes more difficult due to the highest deactivation of the chlorine atom and so vigorous reaction are required for this replacement step. The 2, 4-diamino-6-chloropyrimidine compounds from the second step of substitution that discussed above are used as starting material and reacted either with amines or alkoxides. The result of this reaction is displacement of chloro group at position 6 of pyrimidine core with a new moiety. A further purification is still needed to afford a pure 2,4,6-trichloropyrimidies [**Scheme 3.11**].

Scheme 3.11:-General strategy for the synthesis of 2,4,6-trichloropyrimidine derivatives.

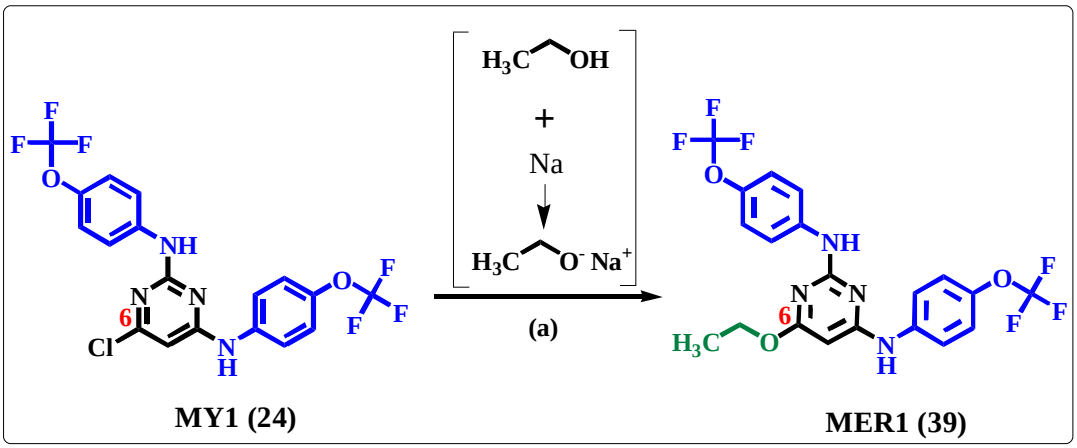


Reagents and conditions: **a)** solvent, reflux hours, extraction and evaporation of solvent and re-crystallization.

3.1.3.1. Synthesis of 6-ethoxy-N2,N4-bis(4-(trifluoromethoxy)phenyl)pyrimidine-2,4-diamine {MER1(39)}

The starting reagent that used in this reaction was a symmetrical 2,4-diamino compound **MY1 (24).** Ethanol is also used as reagent and solvent. This reaction includes the displacement of chlorine atom at 6-position by ethoxide group ($CH_3CH_2O^-$). By reacting ethanol with sodium metal a sodium ethoxide is formed *in situ* and acts as nucleophile to make a nucleophilic attack on the carbon carrying the third chlorine atom. 1.2 moles of sodium is used per mole of 2,4-diamino-6-chloropyrimidine. The reaction of compound **(39)** took about 90 hours and obtained in 75% yield. The procedure of the compound depicted in **[Scheme 3.12]**.

Scheme 3.12: Representative procedure for the synthesis of **39**:

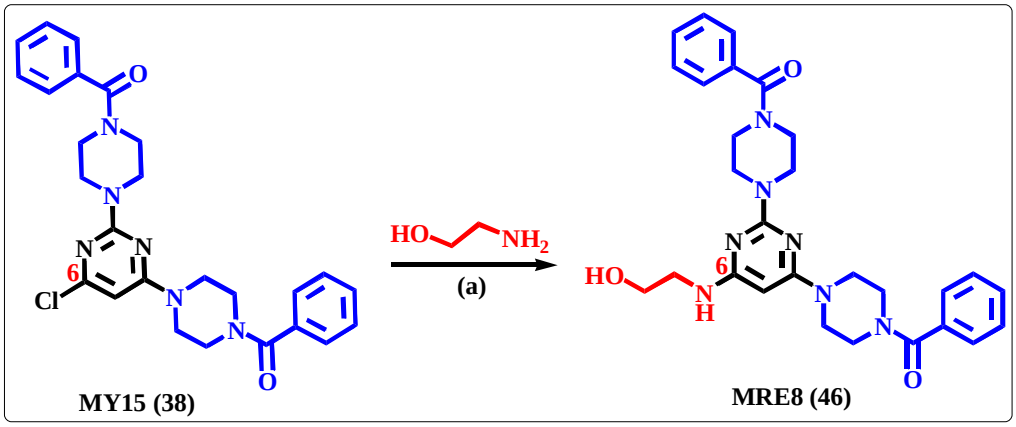


Reagents and conditions: a) EtOH, Sodium metal, stirring 90 hours for **(39)**, extraction, and evaporation.

3.1.3.2. Synthesis of {4-[4-(4-Benzoylpiperazin-1-yl)-6-(2-hydroxyethylamino)-pyrimidin-2-yl]-piperazin-1-yl}-phenylmethanone MRE8 (46)

The starting reagent that used in this reaction is also a symmetrical 2,4-diamino compound **MY15 (38)** that previously prepared. The reaction was performed in dioxane under basic conditions using K_2CO_3 by using ethanolamine **(12)** as a substituent for replacing the third chlorine atom at the 6-position, the reaction undergo nucleophilic substitution. Extraction and the re-crystallization were performed to purify the 2,4,6-triaminopyrimidine compound **(46)** with a yield 33%. The procedure of prepared compound depicted in **[Scheme 3. 13]** below.

Scheme 3.13: Representative procedure for the synthesis of {4-[4-(4-Benzoylpiperazin-1-yl)-6-(2-hydroxyethylamino)-pyrimidin-2-yl]-piperazin-1-yl}-phenylmethanone **(46)**

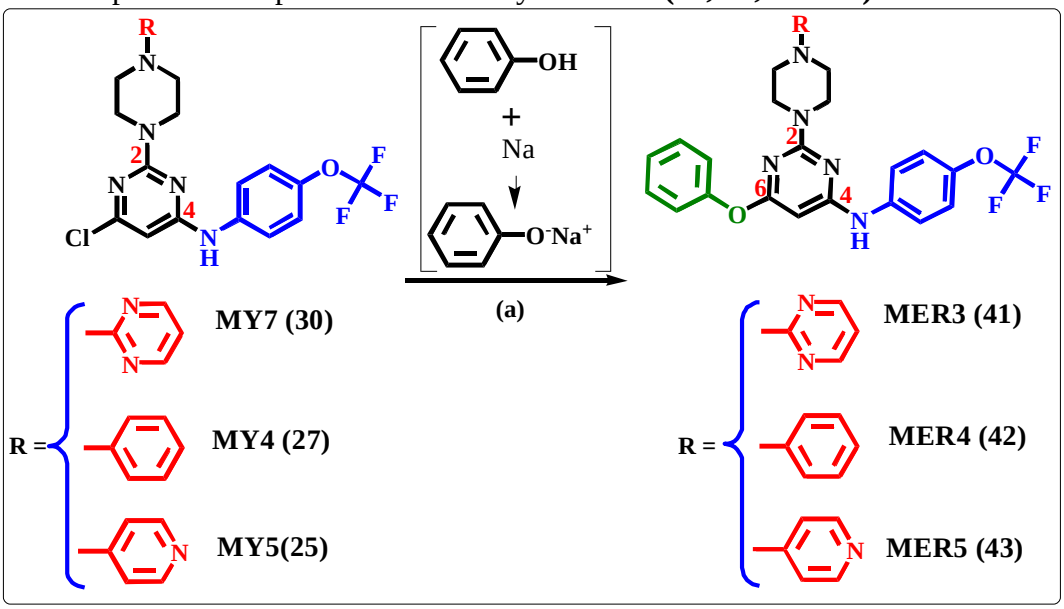


Reagents and conditions: **a)** K_2CO_3, dioxane, stirring at reflux 100 ˚C for 25 hours, extraction.

3.1.3.3. Synthesis of 2,4,6-trisubstituted analogues (41-43)

Asymmetric 2,4,6-trisubstituted analogues **(41), (43), (44)** were synthesized using the intermediates **(28), (30), (27)** respectively. Phenol was converted into a nucleophilic form with sodium metal in dried THF; a sodium phenoxide is formed *in situ* and acts as nucleophile to make a nucleophilic attack on the carbon carrying the third chlorine atom. Extraction and re-crystallization performed to purify the compounds **(41), (42), (43)** with a yield 29%, 33%, 21% respectively. The procedures of the three compounds depicted in **[Scheme 3.14]** below.

Scheme 3.14: Representative procedure for the synthesis of **(42, 43, and 44)**:

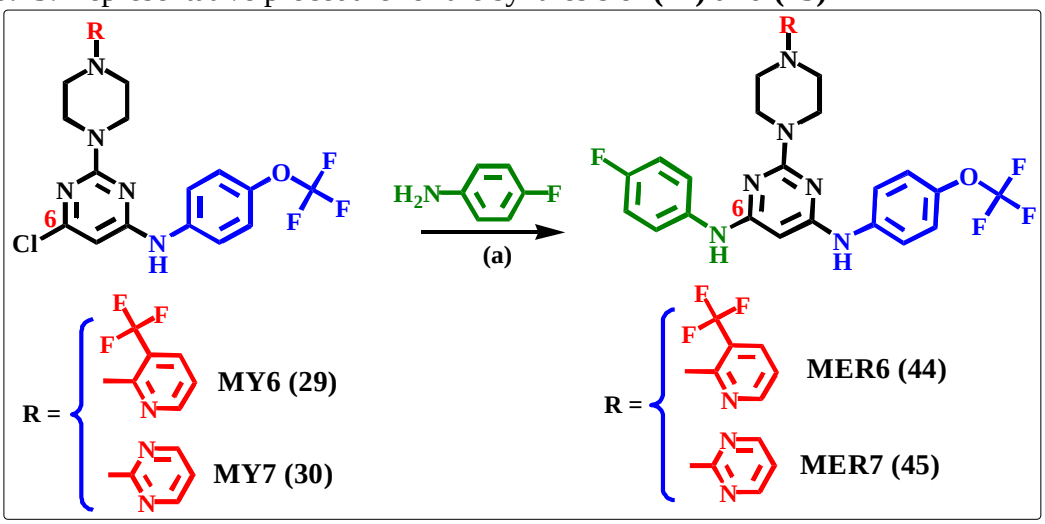


Reagents and conditions: **a)** phenol, sodium metal, THF dry. Stirring at reflux for about 100-120 hours, extraction, and re-crystallization using hexane.

3.1.3.4. Synthesis 2,4,6-triamiopyrimidine derivatives of (44-45)

In these reactions 4-fluroaniline **(2)** was used as a substituent for replacing the third chlorine atom at the 6- position, the reactions undergo nucleophilic attack substitution under acidic conditions using 37% HCl. The precursors **(29), (30)** were used as precursors to give the 2,4,6-triaminopyrimidine compounds **(44), (45)** in 20%, 26% yield respectively [**Scheme 3.15**]. The reactions were stirred at reflux for 40-48 hours until the completion.

Scheme 3.15: Representative procedure for the synthesis of **(44) and (45)**



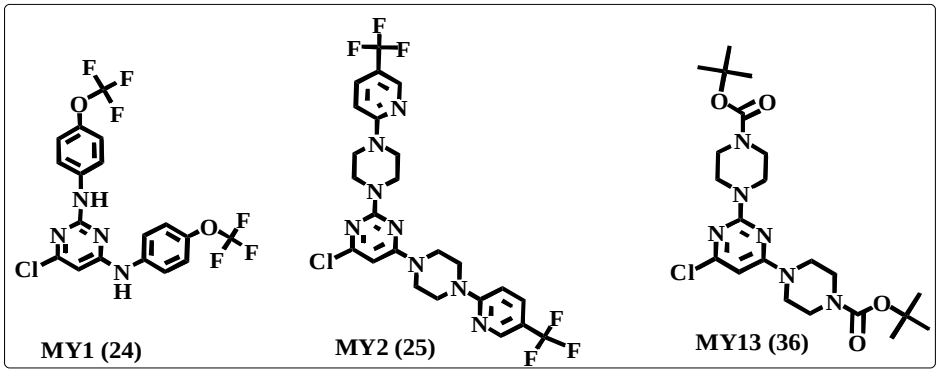
Reagents and conditions: **a)** 37% HCl, dioxane, stirring at reflux at 100 ˚C for 40 hours for **(44)**,48 hours for **(45)**, extraction, re-crystallization.

Conclusion of the Synthetic Chemistry Part

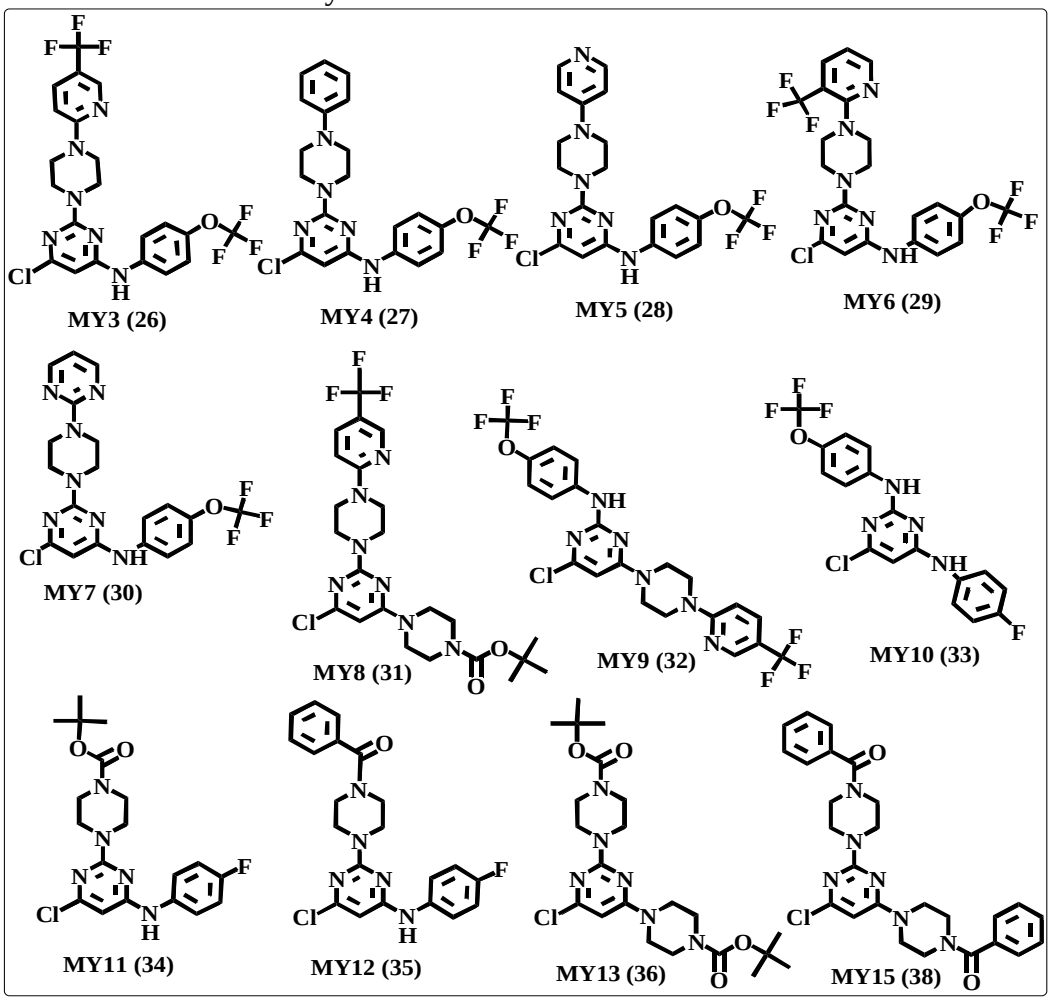
Disubstituted pyrimidine derivatives

A set of 15 compounds of 2,4-diaminosubstituted pyrimidines were prepared through this research. Three of them are symmetrical derivatives **(Figure 3.1(a)** and the other 12 are asymmetrical **(Figure 3.1 (b)**. All these newly synthesized compounds were purified and characterized and some of them were tested for their biological activity.

Figure 3.1 : Disubstituted pyrimidine Derivatives **(a)** Symmetrical Disubstituted Pyrimidines Derivatives



(b) Asymmetrical Disubstituted Pyrimidines Derivatives

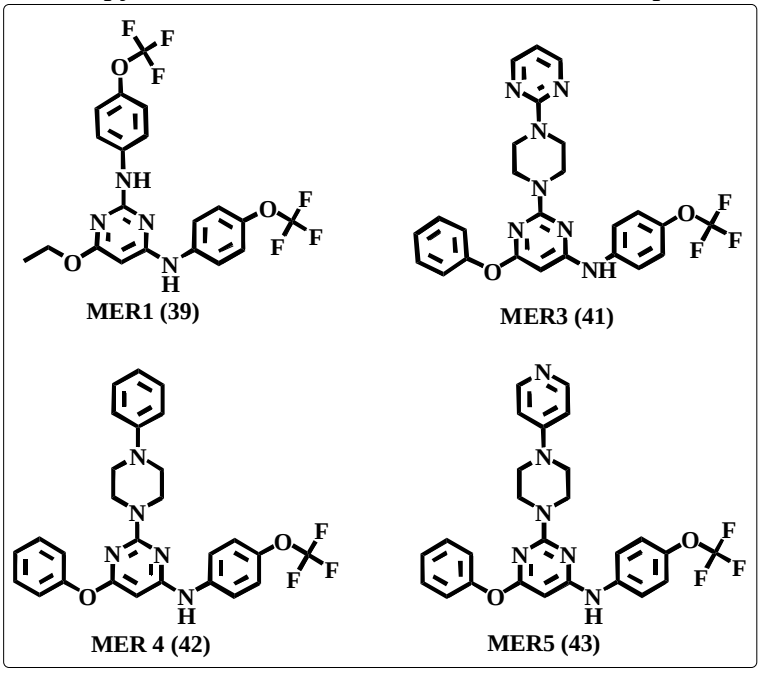


Trisubstituted pyrimidine derivatives

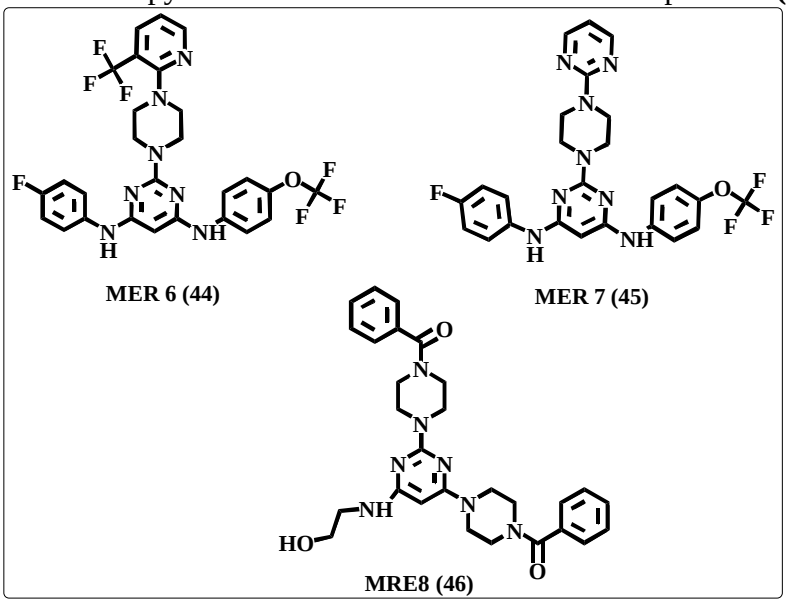
A set of 7 compounds of 2,4,6-trisubstituted pyrimidines were prepared through this research as a final compounds. Four of them are with alkoxide substituent at 6-position (**Figure 3.2(a))** and the other three are with amino group at 6-position (**Figure 3.2 (b))**. All of these newly synthesized compounds were purified and some of them are still need more characterization.

Figure 3.2: 2,4,6-Trisubstituted pyrimidine Derivatives

a) 2,4,6-trisubstituted pyrimidines with alkoxide substituent at 6-position **(39, 41,42,43)**



b) 2,4,6-trisubstituted pyrimidines with amine substituent at 6-position **(44, 45, 46)**



3.2. Biological Activity of the Synthesized compounds

The main aim of the study is to synthesize 2,4,6-trisubstituted pyrimidine derivatives to act as Coactivator Binding Inhibitors (CBIs) for estrogen receptor signaling as a alternative approach for breast cancer treatment. However, intermediates that were synthesized down the road toward 2,4,6-trisubstituted derivatives i.e. 2,4-diamiopyrimidines were assessed for their biological activity against Acute Myeloid Leukemia (AML). The rationale behind this was our awareness that 2,4-disubstituted pyrimidines were known as potential kinase inhibitors. Aggressive AML is known as FLT3-ITD [FMS-like tyrosine kinase 3) internal tandem duplication (ITD)) receptor tyrosine kinase mutant driven. Hence the decision to evaluate the activity of the synthesized compounds in Acute Monocytic Leukemia (AML) cells.

***In vitro* Assessment of Cellular Activity in Acute Myeloid Leukemia (AML) Cell Line:**

To evaluate the cellular activity of the synthesized compounds, acute monocytic leukemia (Molm-13) cell line were used. This cell line had been stably transfected by sh-RNA (empty vector) and sh-p53 RNA (sh-p53). (shRNA) a short hairpin RNA is an RNA sequence that can be engineered to suppress the expression of desired genes via RNA interference (RNAi). [130] shRNAs have been often applied for *in vivo* gene silencing. They have been delivered using viral vectors or being directly injected into fertilized oocytes for making transgenic mice [131]

(Molm-13 shp-53) are p53 knocked down Molm-13 cells that generated by retroviral transfection for stable expression of shRNA against p53 using the pRETRO SUPER-p53 vector. p53 is a gene that produces a protein, called p53 protein that can detect abnormal cells, and then start to kill them to prevent their reproduction, therefore preventing tumor formation. [132, 133] p53 a tumor suppressor gene in a wide spectrum of human cancers that is frequently mutated in up to 50% of breast, colon, lung, liver, prostate, bladder, and skin cancer. Most of these mutations end are defined as loss of function of p53.[134] For the biological assessment of the inhibitory effect of the synthesized compounds Molm-13 cell lines were used.

To evaluate the role of p53 in growth inhibition two forms of Molm-13 cells were used. In one case cells were transfected time with empty vector and in the other the needed concentration of the relevant compound was added to cells tranfected with sh-p53 RNA (sh-p53). The viability of the cells was determined using WST-1 assay. (**Figure 3.3**) below shows the nuclear staining using this assay for compound MY3 **(26).**

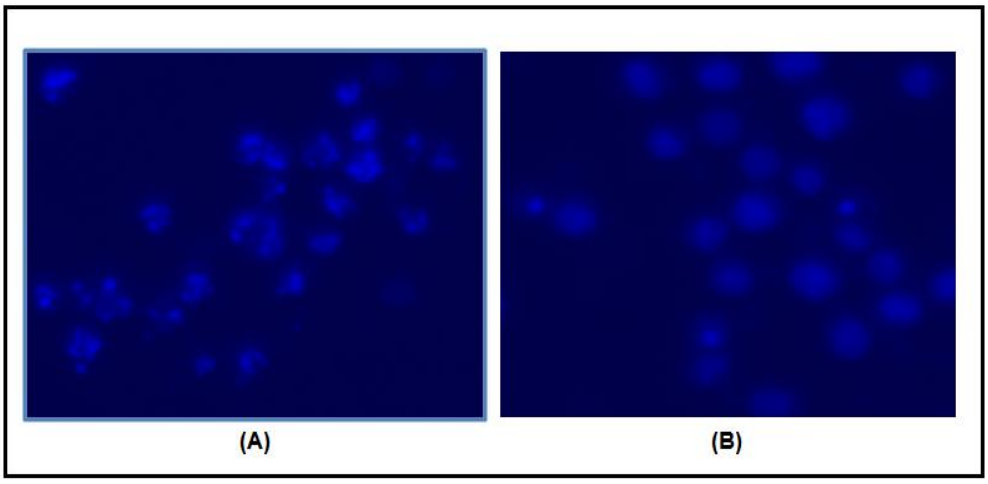


Figure 3.3: **A)** nuclear staining of HL60 cells using WST-1 assay after treatment with 10μM of **MY3 (26)** for 24 hours; **B)** nuclear staining of Molm-13 cells using WST-1 assay after treatment with 10μM of **MY3 (26)** for 24 hour.

Molm-13 growth Inhibition and SAR Studies

The structure-activity relationship (SAR) that could be drawn from the (IC_{50} values, **Table 3.1**) Indicated that the potency was sensitive to steric and electronic factors of the substituent residing at C-2 and C-4 positions of the central pyrimidine ring. The tested compounds have show weak to moderate activity against Molm-13 (empty vector) [IC_{50} = 8.3 to 33.7 μM] and Molm-13 (sh-p53) [IC_{50} = 9.3 to 24.83 μM]. Compound **(35)** was relatively more potent than others with IC50 (8.3 μM and 9.3 μM respectively) while **(38)** is the least potent with IC_{50} (33.57 μM and 24.83 μM).

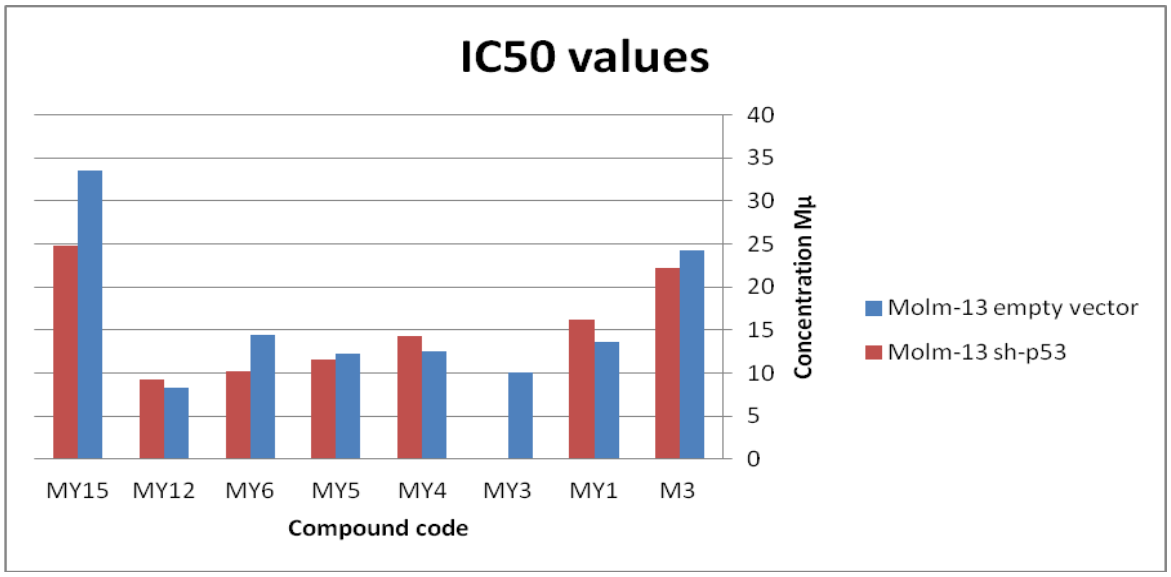
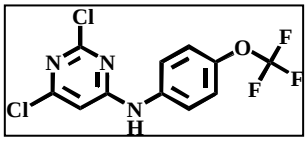
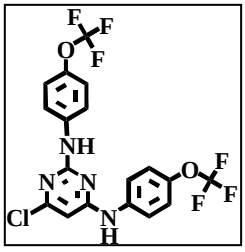
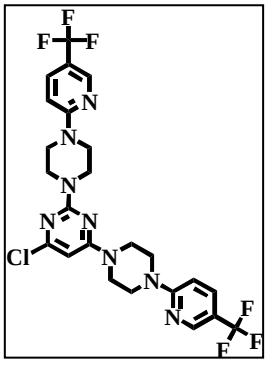
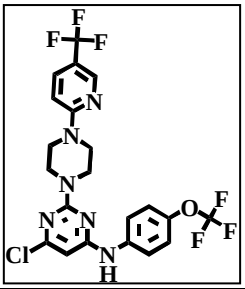
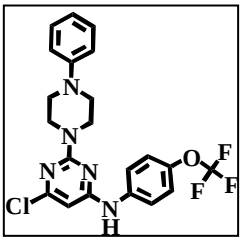
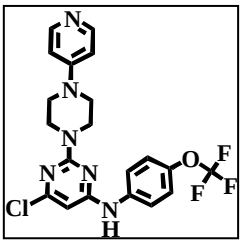
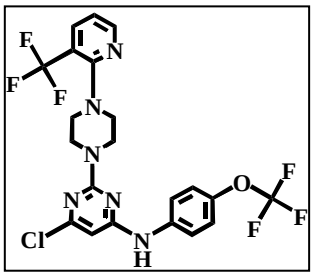
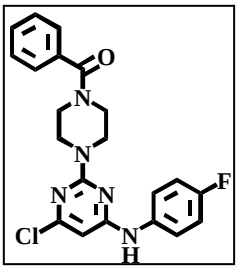
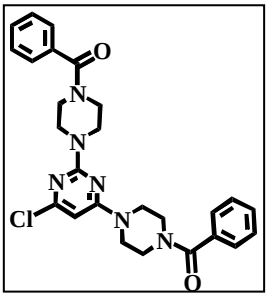


Figure 3.4: IC_{50} values for the compounds (**M3 (17)**, **MY1 (24)**, **MY3 (26)**, **MY4 (27)**, **MY5 (28)**, **MY6 (29)**, **MY12 (35)**, and **MY15 (38)**) in Molm-13 cell line.

Table 3.1: Structural activity relationship and IC_{50} values of the compounds (**17, 24, 25, 26, 27, 28, 29, 35, and 38**)

Number	Code	Structure Compound	IC_{50} (in μM) Molm-13 (empty vector)	IC_{50} (in μM) Molm-13 (sh-p53)
17	**M3**		**24.27**	**22.18**
24	**MY1**		**13.61**	**16.18**
25	**MY2**		**NA**	**NA**

26	**MY3**		**10**	**(not tested in this cell type)**
27	**MY4**		**12.47**	**14.25**
28	**MY5**		**12.28**	**11.58**
29	**MY6**		**14.48**	**10.23**
35	**MY12**		**8.3**	**9.3**
38	**MY15**		**33.75**	**24.83**

NA = Not active

The activity of the compound **MY3 (26)** was evaluated against two cell lines. The first is the Molm-13 and the second was HL-60 (human promyelocytic leukemia cells). The second cell line was derived from a 36-year-old woman with acute promyelocytic leukemia at the National Cancer Institute and is predominantly a neutrophilic promyelocyte (precursor). Results have showed that the IC_{50} of the **MY3 (26)** was 10 µM in the Molm-13 and was 10.3 µM in HL-60 cells. These results indicate that **MY3 (26)** exerts similar activity in the both cell lines and that p53 not involved in the mechanism of action of **MY3 (26)**. From the graph shown in **(Figure 3.5)** a dose dependent effect of **MY3 (26)** was noticed that as the concentration of **MY3 (26)** increased the viability of the cells decreases**.** Molm-13 cells were treated for 24 hour at 10 µM concentration and viability of the cells was determined using WST-1 assay. The nuclear staining results revealed that the dye intensity decreases in the cells that subjected to 10 µM of **MY3 (26) (Figure 3.3)** indicating that this compound has an inhibition activity on the cells.

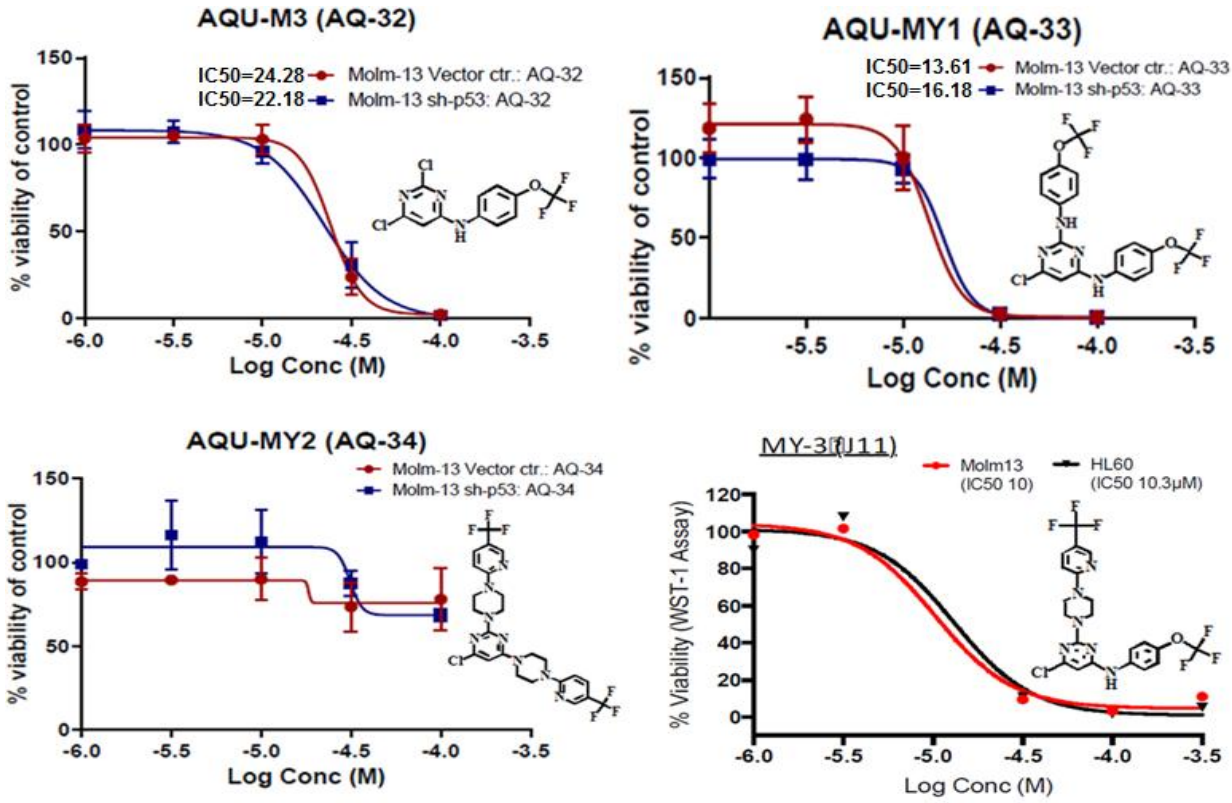


Figure 3.5: Concentration-response effect of **M3 (17)**, **MY1 (24)** and **MY2 (25)**, **MY3 (26)** on cancer cell culture: cell viability was measured by WST-1assay. Data are expressed as the mean ± SD of two independent experiments.

To understand the effect of character of the substituent and the position on the pyrimidine core the IC_{50} of **MY3 (26)** was compared to other derivatives. The compound **MY3 (26)** with a 4-trifluromethoxyaniline group at 4-position and with 1-(5-trifluoromethyl-pyridin-2-yl)-piperazine at 2-position have a moderate IC_{50} values about 10 μM. To identify which among the two substituents (trifluromethoxyaniline or 1-(5-trifluoromethyl-pyridin-2-yl)-piperazine) is more important for the activity the symmetrical analogues were synthesized. When the 2- and 4-position of pyrimidine were substituted, with 4-trifluromethoxyaniline group in **MY1 (24)** the compound still have a moderate potency with IC_{50} 13 μM but lower than **MY3 (26) (Figure 3.5)**. The presence of the two 4-trifluromethoxyaniline groups decrease the activity than in the case of using one group of 4-trifluromethoxyaniline in **MY3 (26).** When the 2- and 4-positions of pyrimidine were substituted with 1-(5-trifluoromethyl-pyridin-2-yl)-piperazine, the resulted compound **MY2 (25)** showed a highly drop in the potency and no significant inhibition had been noticed. This result confirms the importance of presence of one trifluromethoxyaniline group on the pyrimidine core where when the trifluromethoxy was excluded from the compound **MY2 (25)** the potency decreesed in a remarkable values **(Figure 3.5)**. Comparision between the structures and the potency of these three compounds **(MY3 (26), MY1 (24), MY2 (25))** emphasize that the 4-trifluromethoxyaniline should be maintained as a substituents at 4- positions and that the variation should include the substitutions at 2-position. After showing the selective importance of at least one trifluromethoxyaniline at 4-position of the pyrimidine ring, we wanted to probe the ability of other piperazine derivatives at 2-position to affect the potency

To develop a structure–activity relationship (SAR) around the compound **MY3 (26).** A series of MY3 analogues had been synthesized by keeping the 4-pyrimidine substitution pattern with the trifluoromethoxyaniline group, and adding several structural moieties at the C-2 of the pyrimidine ring using different piperazinyl-derivatives. The analogues have been synthesized, purified using

chromatography techniques, characterized by ^{1}H-NMR. Three of these analogues had been biologically tested and investigated for having activity on Molm-13 cancer cells. Results of the tested compounds demonstrated that compounds containing phenyl piperazine such as **MY4 (27)**, 1-4 pyridypiperazine such as **MY5 (28)**, 1-(3-trifluoromethyl-pyridin-2-yl)-piperazine such as **MY6 (29)** at the 2-position of the pyrimidine ring exhibit moderate biological activity. **MY4 (27)** have lower potency than **MY3 (26).** It differs from **MY3 (26)** in having phenyl ring instead of pyridine ring with attached CF_3 group to the ring. The compounds **MY5 (28)** and **MY6 (29)** have a lower potency than **MY4 (27).** These two compounds contain N-heteroaromatic ring (pyridine ring). It became evident that there is a necessary relationship between the presence of N-heteroaromatic ring attached to the piperazine ring and the potency, where the potency decreased in the case of N-heteroaromatic ring compared with the case of phenyl ring. We rationalized that the importance of the phenyl ring was most likely based on its ability to make a hydrophobic interaction and fit deeply in the pocket while the N-heteroatom has the ability to act as a hydrogen-bond donor. Compound **MY6 (29)** which exactly contains the same substituent groups that present in **MY3 (26)** but with a small structural changes of the CF_3 substituents on the N-heteroaromatic ring at 3-position instead of 5-position in **MY3 (26).** This small difference of branched CF_3 position had influenced the binding affinities and potency of this compound. **(Figure 3.6)**

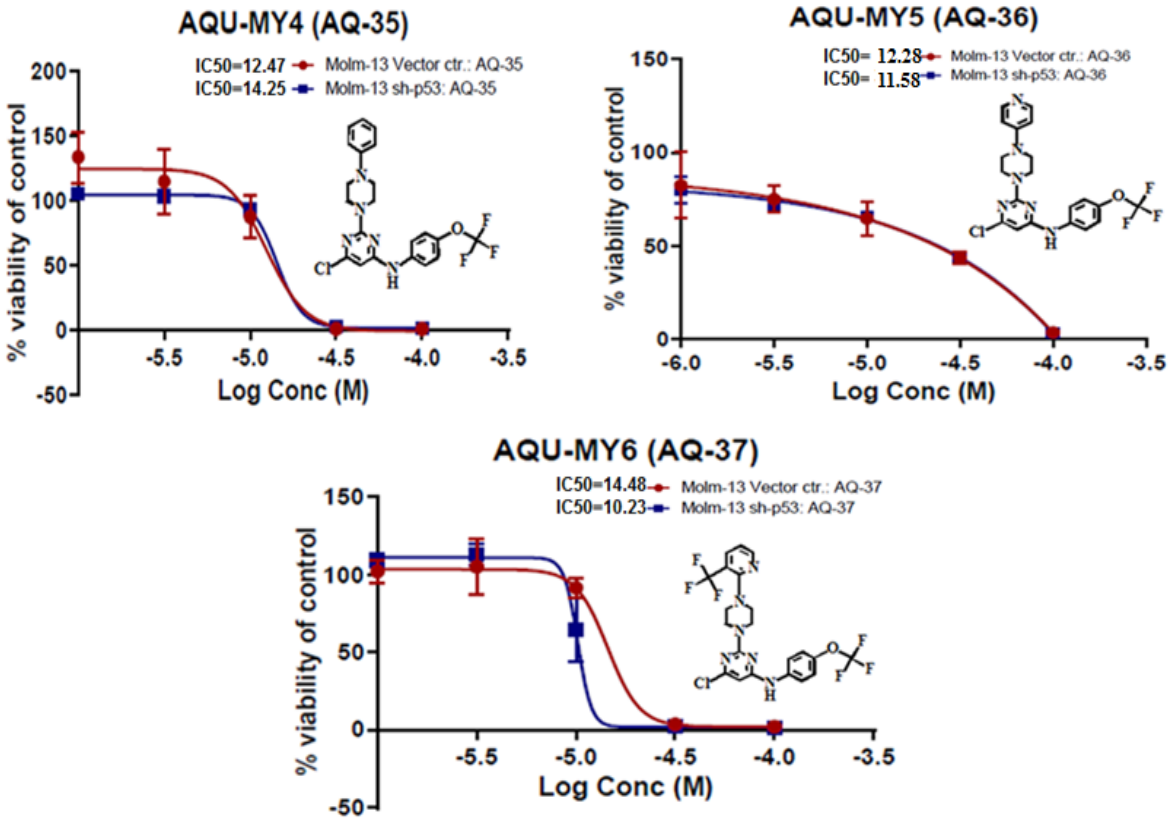


Figure 3.6: Concentration-response effect of **MY4 (27)**, **MY5 (28)** and **MY6 (29)** on cancer cell culture: cell viability was measured by WST-1assay. Data are expressed as the mean ± SD of two independent experiments.

The compound **MY12 (35)** with a 4-fluroaniline group at 4-position and with benzoylpiperazine at 2-position have the best IC_{50} values about 8.3 μM. Comparision of activities of this compound with the activity of compound MY15 **(38)** containing two benzoylpiperazine groups at the 2-and 4-positions with IC_{50} Value of 33.75 μM indicated that only one N4-benzoylpiperzinyl- group at 2-position was the causative part of potency increasing and recommended to be retained in future studies **(Figure 3.7)**

The amide bridge in **MY12 (35)** make this compound the most potent one of all compounds containing piperazine derivatives. This bridge might gave the suitable distance for making a hydrogen bond interaction which not allowed in other compounds although they have N-heteroatom and have the ability to make hydrogen bound.

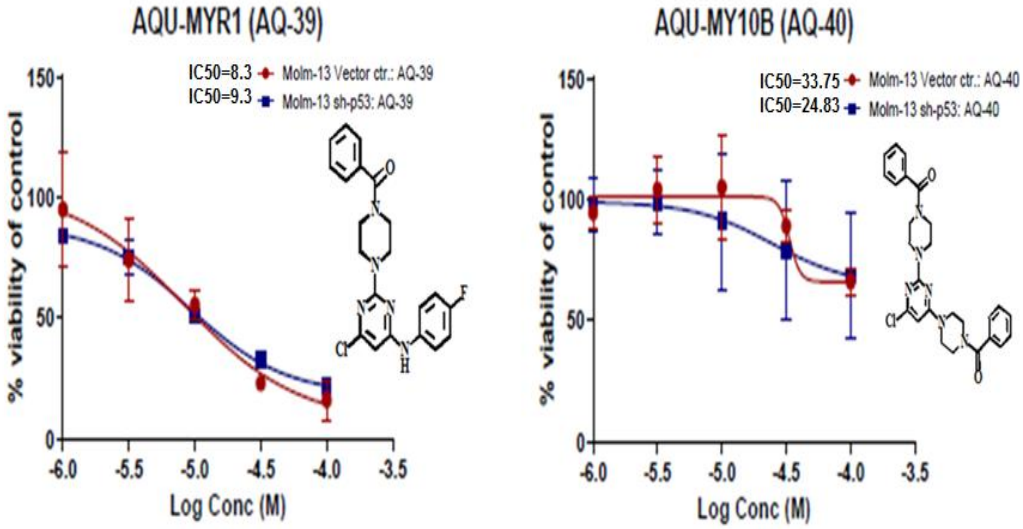


Figure 3.7: Concentration-response effect of **MY12 (35)**, **MY15 (38)** on cancer cell culture: cell viability was measured by WST-1assay. Data are expressed as the mean ± SD of two independent experiments.

The graph inserted below **(Figure 3.8)** showed that at the concentration 1µM MY5 **(28)** was the most active compound while MY4 was the lowest active compound. In other hand the compound MY4**(27)** showed the highest activity at the concentration 31.6 µM with 1% cell viablility while the compound MY15 showed the lowest activity with a 89% cell viability. All the compounds inserted in the graph have a dose dependent effect in killing Molm-13 empty vector cells with the concentration range (31.6µ -100 µ). The compound MY15 **(36)** was the less potent compound of the tested compoundes while the compound MY2 **(25)** had no significant inhibtion and had poor effect in killing and inhibition the cancer cells growth, in other words it was noticed that even at high concentrations (100 µ) the compound seems not working and the cell viability still high. Of all these compounds MY12 **(35)** was the potent one.

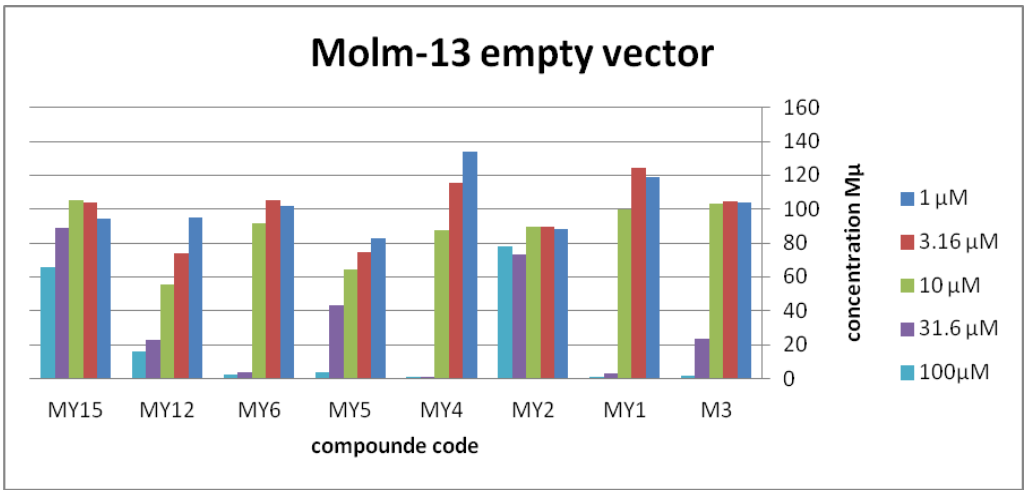


Figure 3.8: Cell vialbilty response of Molm-13 empty vector at different concentrations (1 μM, 3.16 μM,10 μM ,31.6 μM ,100 μM) of different compounds (**M3**, **MY1**, **MY2**, **MY4**, **MY5**, **MY6**, **MY12**, **MY15**).

On (Molm-13 sh-p53) cells, all tested compounds had shown similar activities to those against in Molm-13 empty vector cells **(Figure 3.9)**. In some cases the inactivation of this gene can cause cancer development or other diseases. In acute myeloid leukemia (AML), p53 mutations have been detected in only about 5% of patients, but mutation is recognized as an adverse factor for response to chemotherapy and prognosis. These results demonsrated that the mechanism of action of the tested compounds didn't depend on the p53 pathway to exert their inhibition activity on the tested cells.

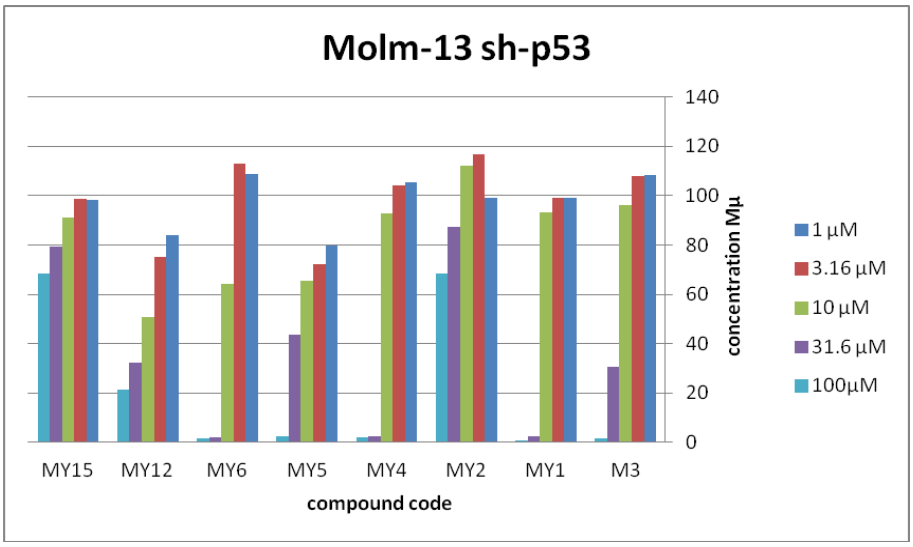
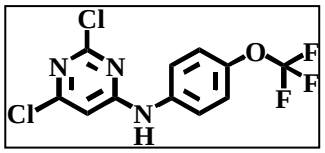
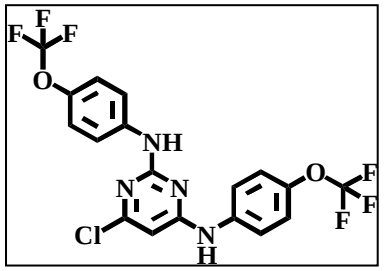
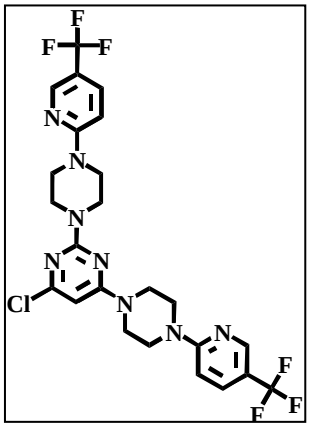


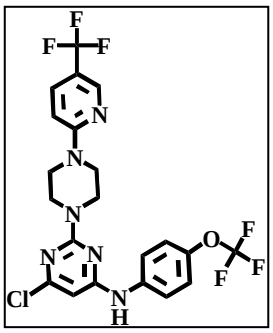
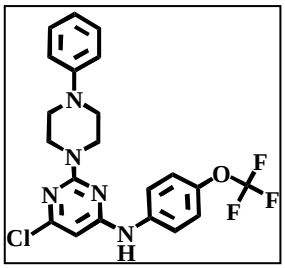
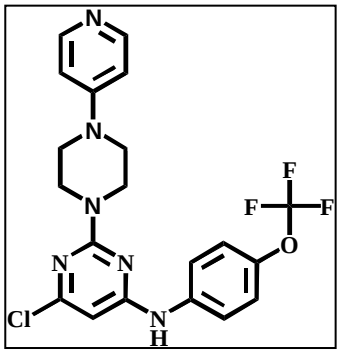
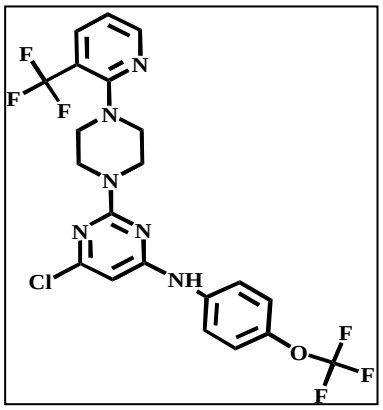
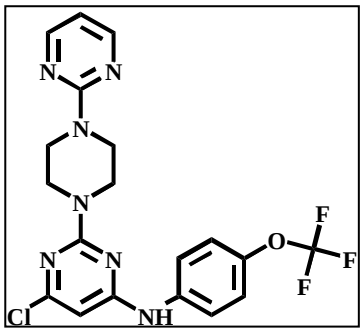
Figure 3.9: Cell vialbilty response of Molm-13 sh-p53 at different concentrations (1 μM, 3.16 μM, 10 μM ,31.6 μM ,100 μM) of different compounds (**M3**, **MY1**, **MY2**, **MY4**, **MY5**, **MY6**, **MY12** , **MY15**).

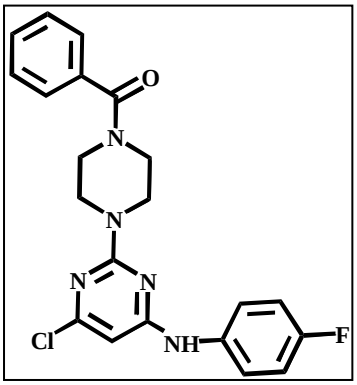
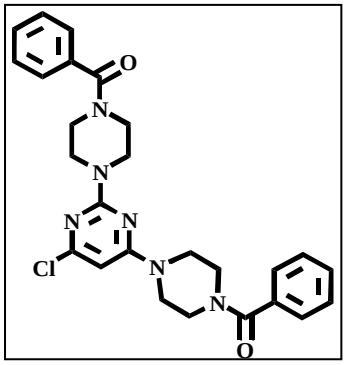
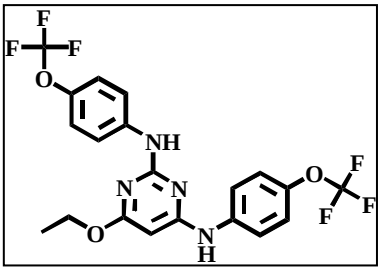
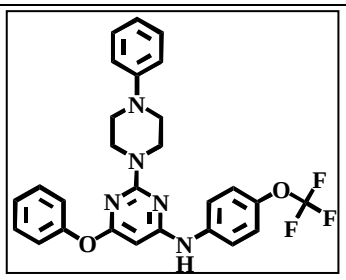
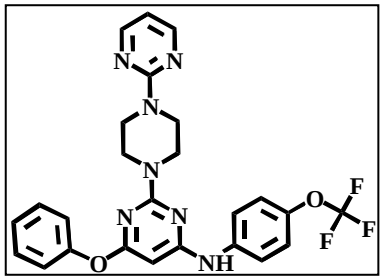
3.3. Computational Chemistry

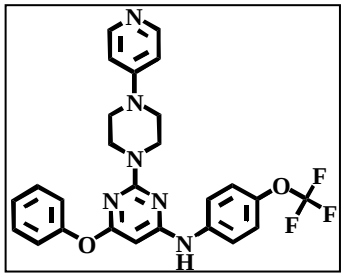
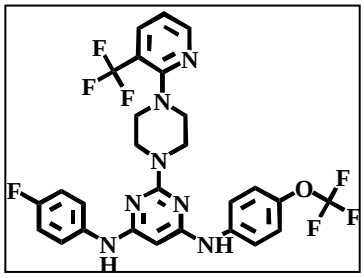
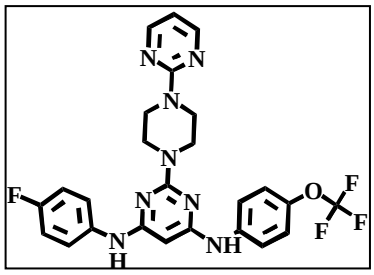
The PASS (Prediction of Activity Spectra for Substances) software had been used to predict the pharmacological effects and biochemical mechanisms of the newly synthesized compounds on the basis of the structural formula of a substance. **(Table 3.2)** shows the predicted activity of some compounds.

Table 3.2: Biological activity spectrum of compound predicted on the basis of SAR

Number	Code	Compound Structure	Mol. Mass	Expected activity
17	**M3**		324.09	-Analagesic, non opioid - Antineoplastic (nonhodgkin's lymphoma) - Antineoplastic (multiple myeloma) - Antipsoriatic -Vasculitis treatment
24	**MY1**		464.75	-Signal transduction pathway inhibitor - Angiogenesis inhibitor -Antieczematic atopic Antineoplastic Transplant rejection treatment
25	**MY2**		572.94	-Hematopotic inhibitor -Antiasthmatic Antineoplastic (nonhodgkin's lymphoma) - Transplant rejection treatment -Anxiolytic

26	**MY3**		518	-Antineurogenic pain -analagesic, non opioid -autoimmune disorders treatment - Transplant rejection treatment - Atherosclerosis teatment
27	**MY4**		449.86	Antieczematic atopic - Antineurogenic -Transplant rejection treatment -Scleroderma treatment -Analagesic, non opioid
28	**MY5**		450.84	- Signal transduction pathway inhibitor - Transplant rejection treatment. -Antieczematic atopic -Antineurogenic
29	**MY6**		518.84	-Analagesic, non opioid -Antineurogenic pain -Atherosclerosis teatment -Autoimmune disorders treatment - Transplant rejection treatment
30	**MY7**		451	-Glycogen synthase stimulant - Nav1.6 sodium channel blocker -Tyrosine-protein kinase BMX inhibitor -potassium channel small-conductance Ca-activated blocker signal transduction pathways inhibitor

35	**MY12**		411.86	-Antieczematic atopic -Transplant rejection treatment. -A nalagesic, non opioid -Hematopotic inhibitor -Scleroderma treatment
38	**MY15**		491	-A nalagesic, non opioid -Hematopotic inhibitor -Scleroderma treatment - Antieczematic atopic -Transplant rejection treatment
39	**MER 1**		474	-signal transduction pathways inhibitor -Tyrosine-protein kinase BMX inhibitor -angiogenesis inhibitor - dihydroorotase inhibitor
41	**MER3**		509	- signal transduction pathways inhibitor - Nav1.6 sodium channel blocker -Tyrosine-protein kinase BMX inhibitor -Glycogen synthase stimulant
42	**MER4**		507	- signal transduction pathways inhibitor -Tyrosine-protein kinase BMX inhibitor - Nav1.6 sodium channel blocker -CC chemokine 6 receptor antagonist

43	**MER5**		508	- signal transduction pathways inhibitor -Tyrosine-protein kinase BMX inhibitor - Nav1.6 sodium channel blocker -Angiogenesis inhibitor -glycogen synthase stimulant
44	**MER6**		594	-Vaniloid antagonist - Tyrosine-protein kinase BMX inhibitor - Nav1.6 sodium channel blocker - signal transduction pathways inhibitor
45	**MER7**		526	-Nav1.6 sodium channel blocker - signal transduction pathways inhibitor - Tyrosine-protein kinase BMX inhibitor - protein kinase inhibitor -glycogen synthase stimulant

Chapter 4

Conclusion

Conclusion

This research project focuses on synthesizing small molecules that mimetic nuclear hormone receptor of (NR) co-activator proteins with special focus on the estrogen receptor alpha (ERα).Such molecules that distrup the (ER)/coactivator proteins may provide an alternative mechanism for blocking estrogen signaling and activity in breast cancer.

The compounds prepared in the current study were done through sequential substitution of the chlorides of 2,4,6-trichloropyrimidine by nucleophiles like amines, alkoxides or aryloxides. The synthesized compounds were purified using chromatography techniques, and characterized by (^{1}H-NMR, ^{13}C-NMR, FT-IR spectroscopy and ESI-MS spectrometry. Some of these compounds were screened for their inhibitory activity against the acute myeloid leukemia cells (Molm-13) cells. The viability of the cells was determined using WST-1 assay.

Initial *in vitro* results of few tested compounds demonstrated that **(35)** and **(26)** exhibited moderate activities against the two forms of Molm-13 cell lines and have a dose dependent response while compound **(25)** showed no significant inhibitory action on the same cell lines.

Chapter Five

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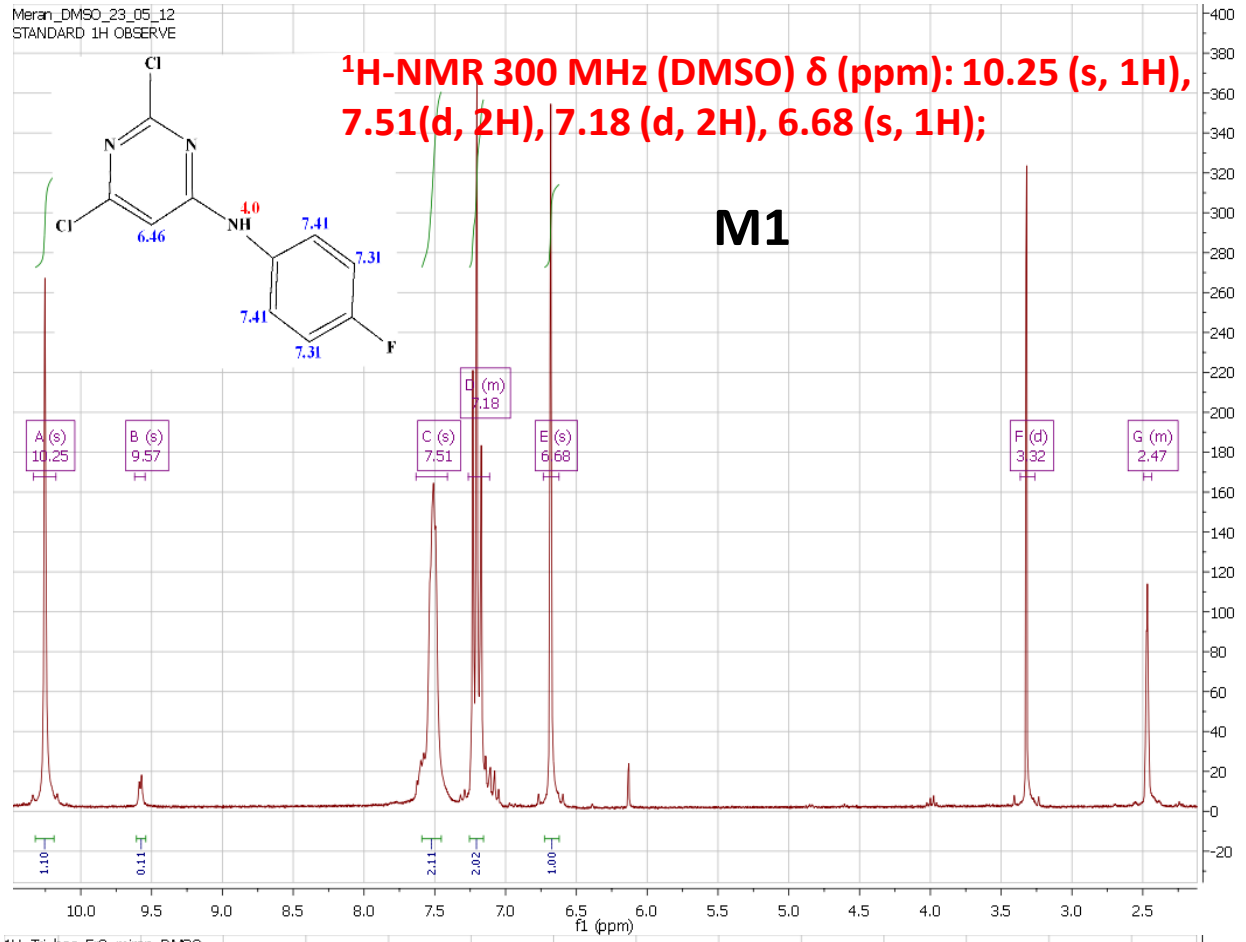
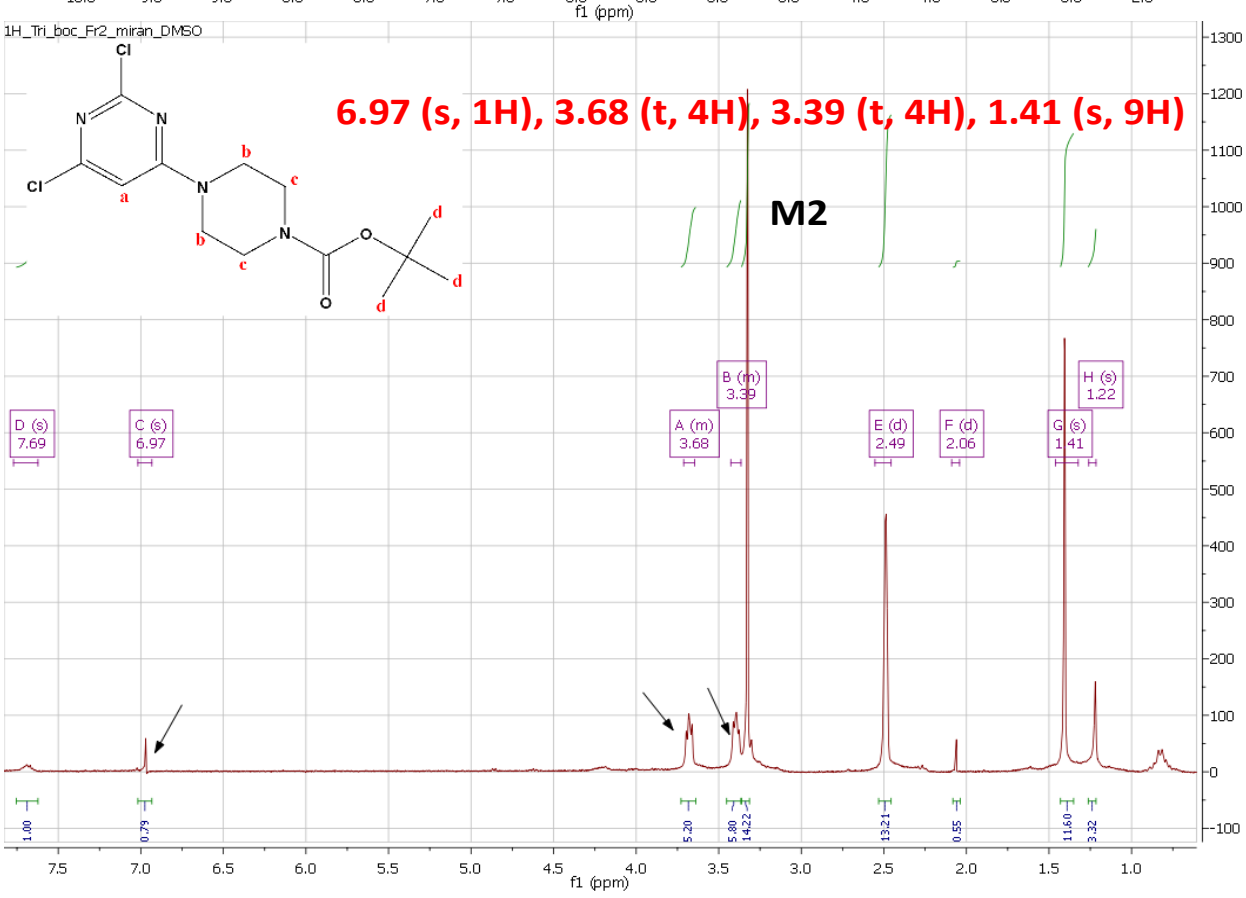
Chapter Six

Appendices



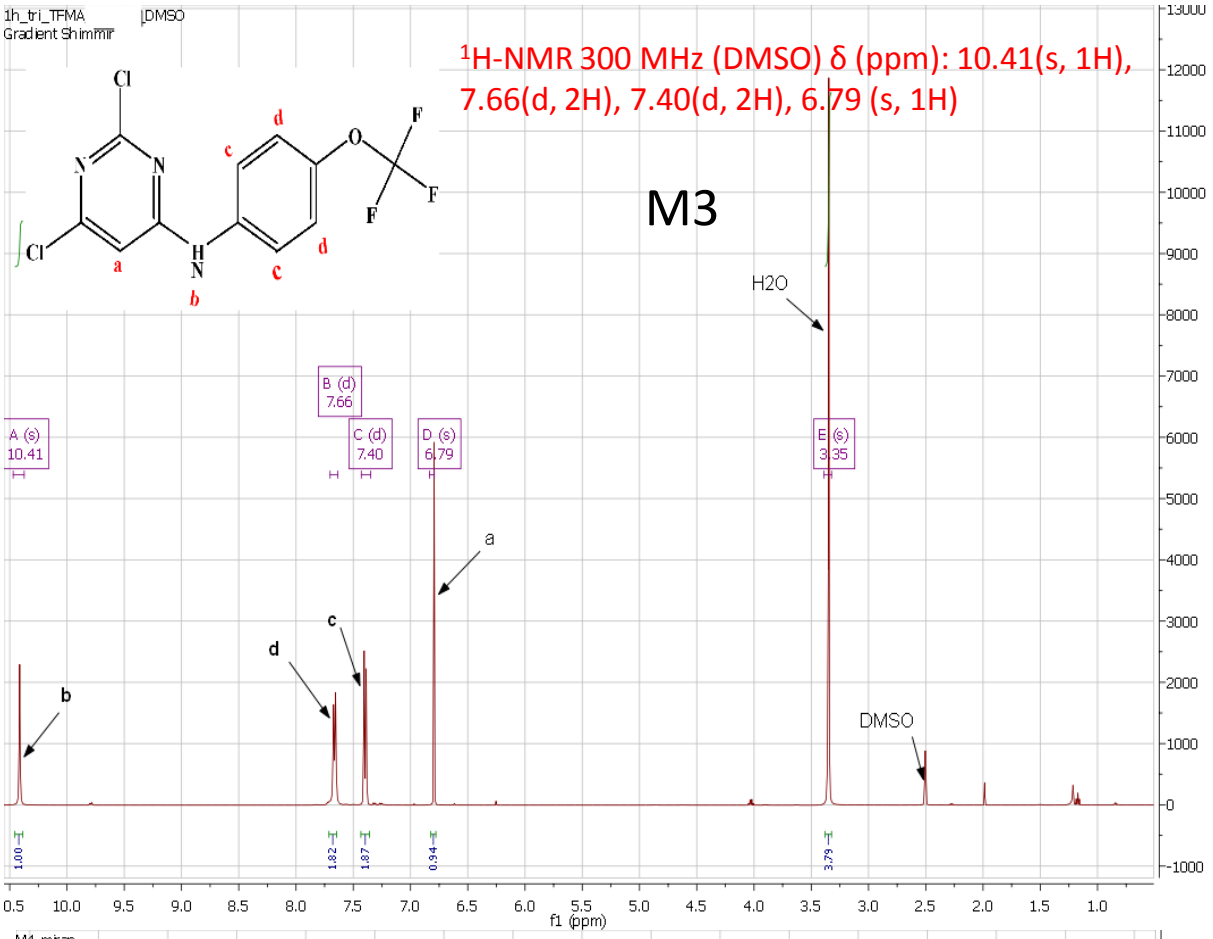
^{1}H-NMR 300 MHz (DMSO) δ (ppm): 10.25 (s, 1H), 7.51(d, 2H), 7.18 (d, 2H), 6.68 (s, 1H);

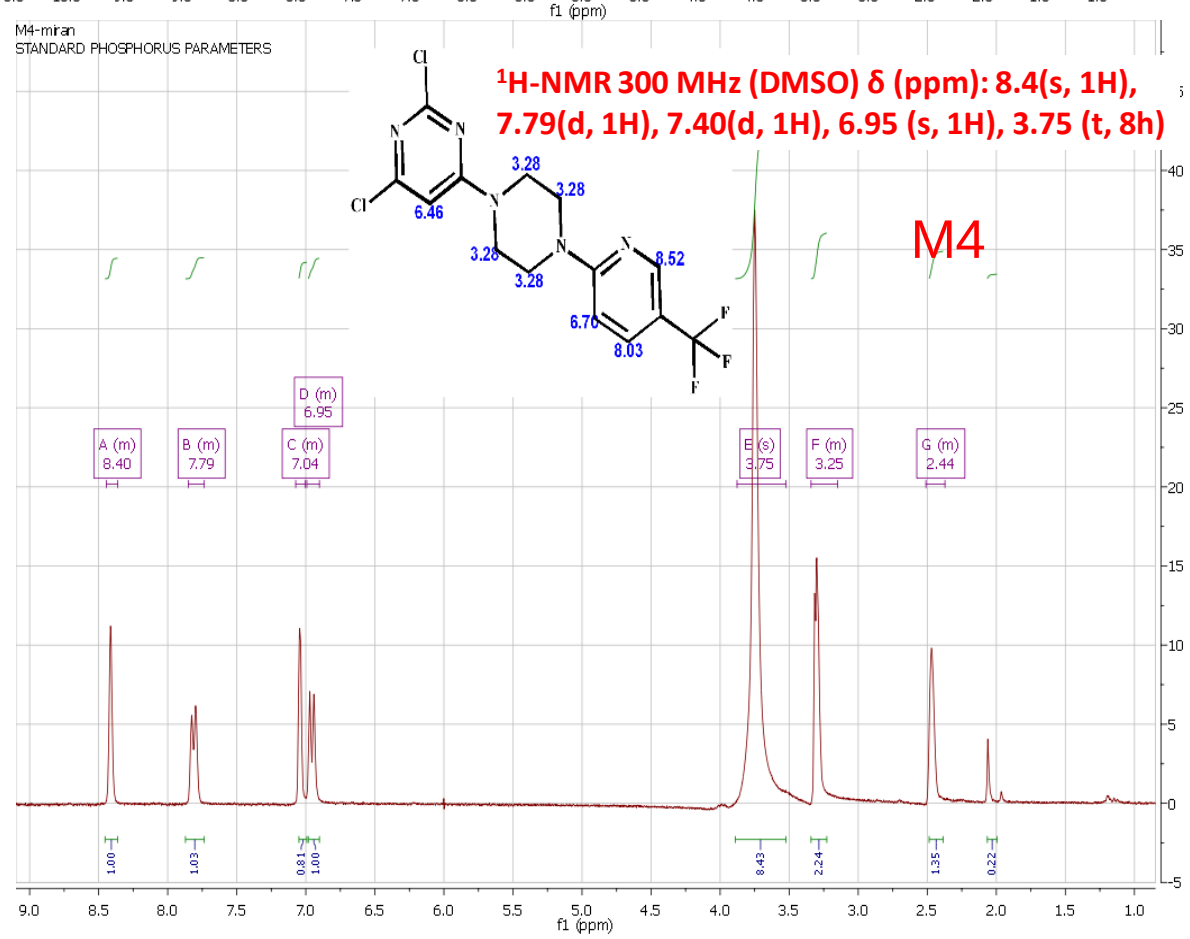
M1

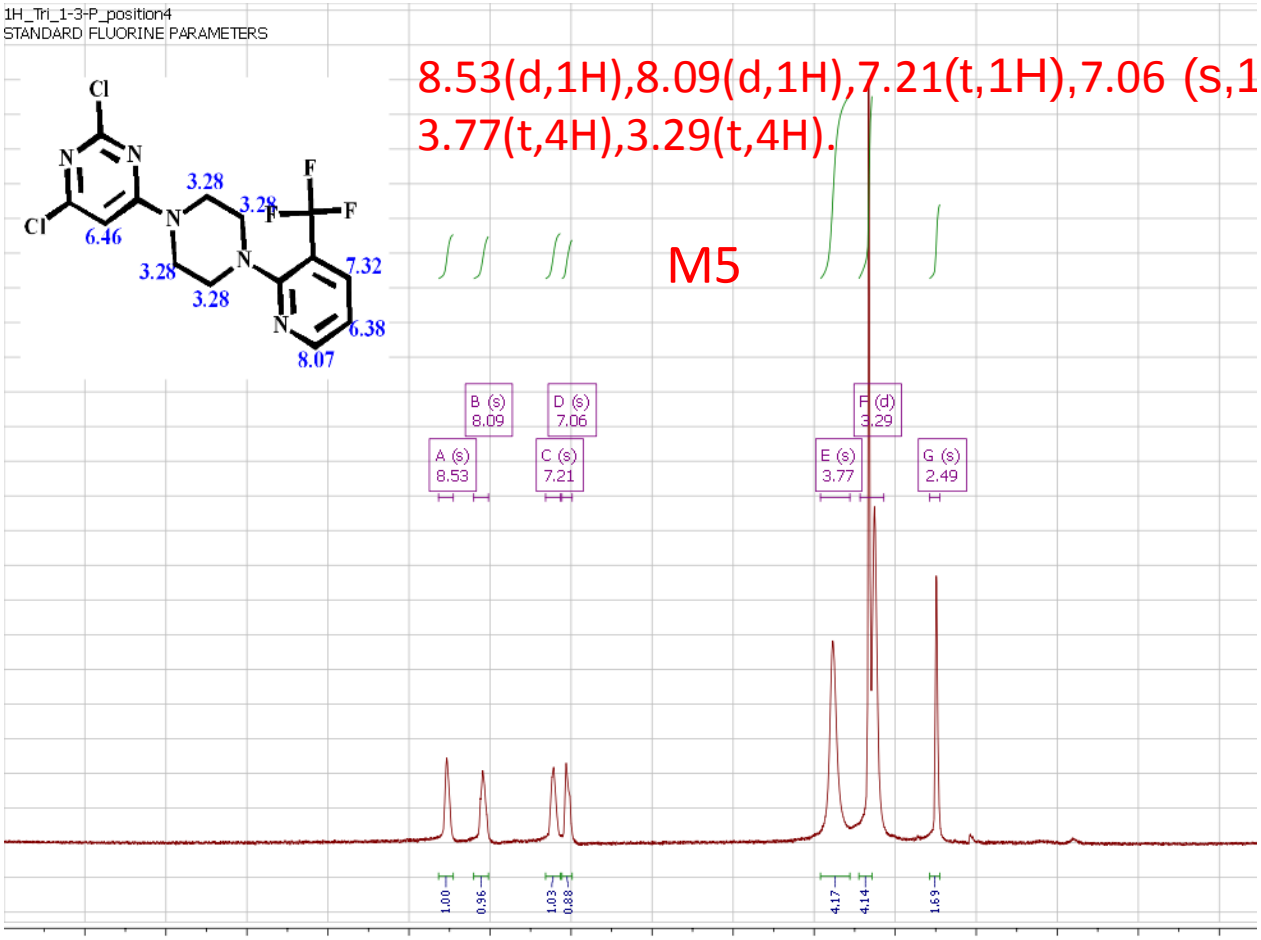


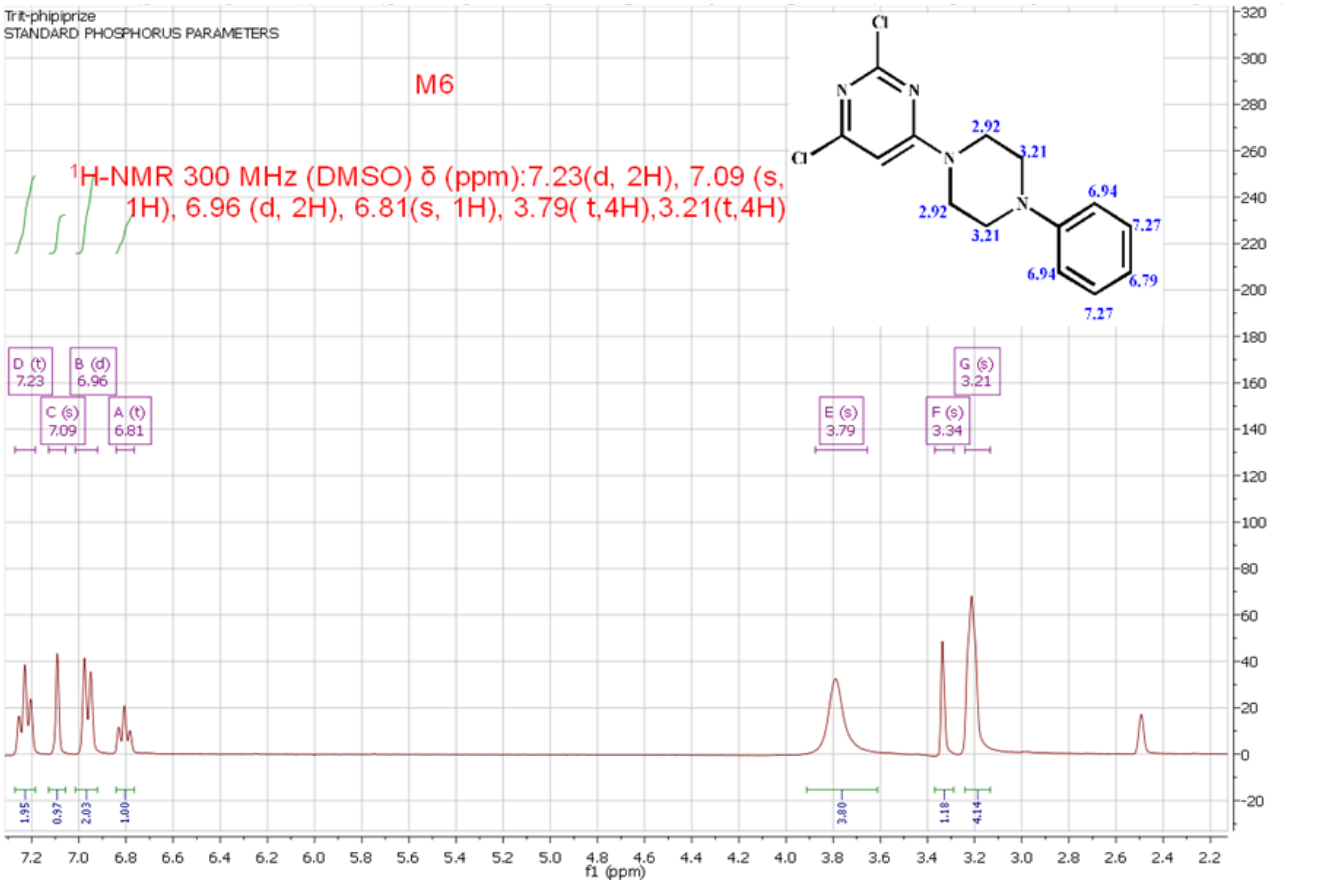
6.97 (s, 1H), 3.68 (t, 4H), 3.39 (t, 4H), 1.41 (s, 9H)

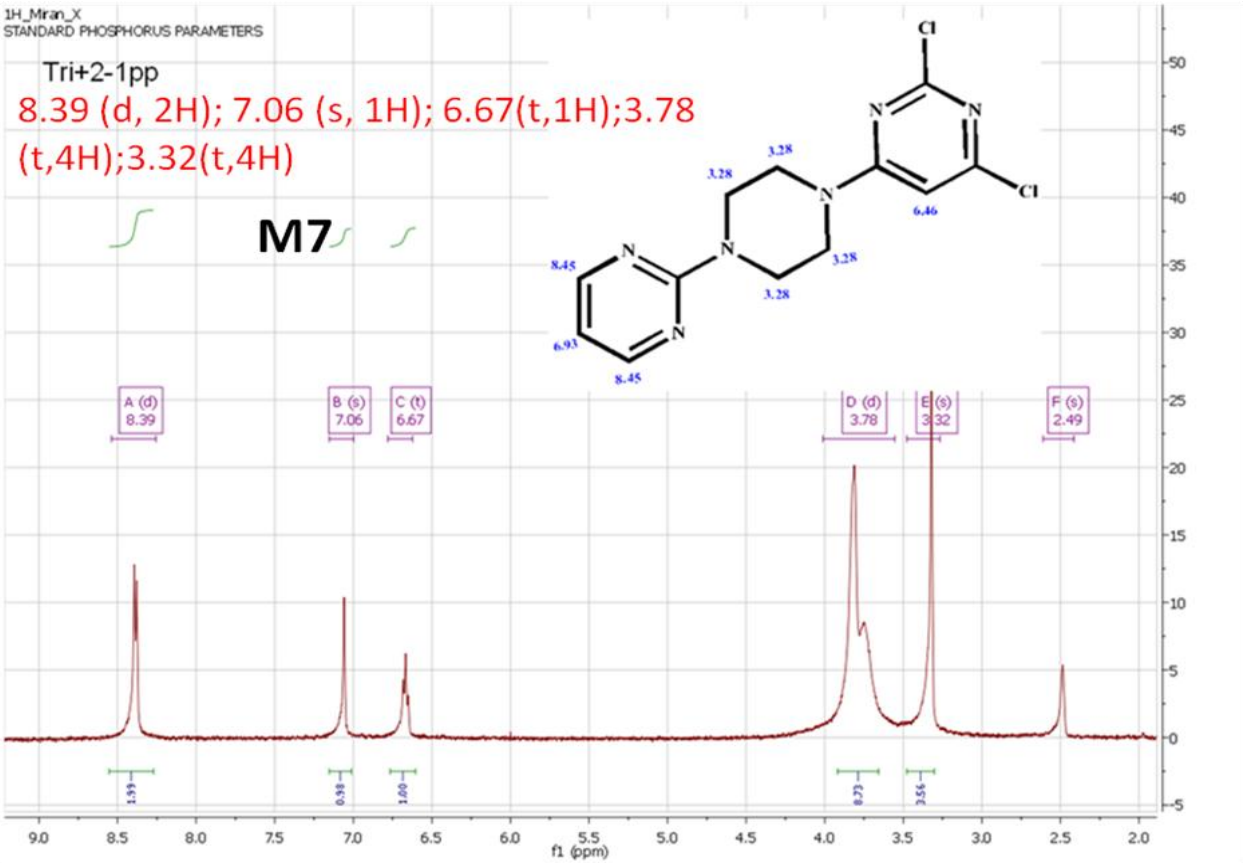
M2

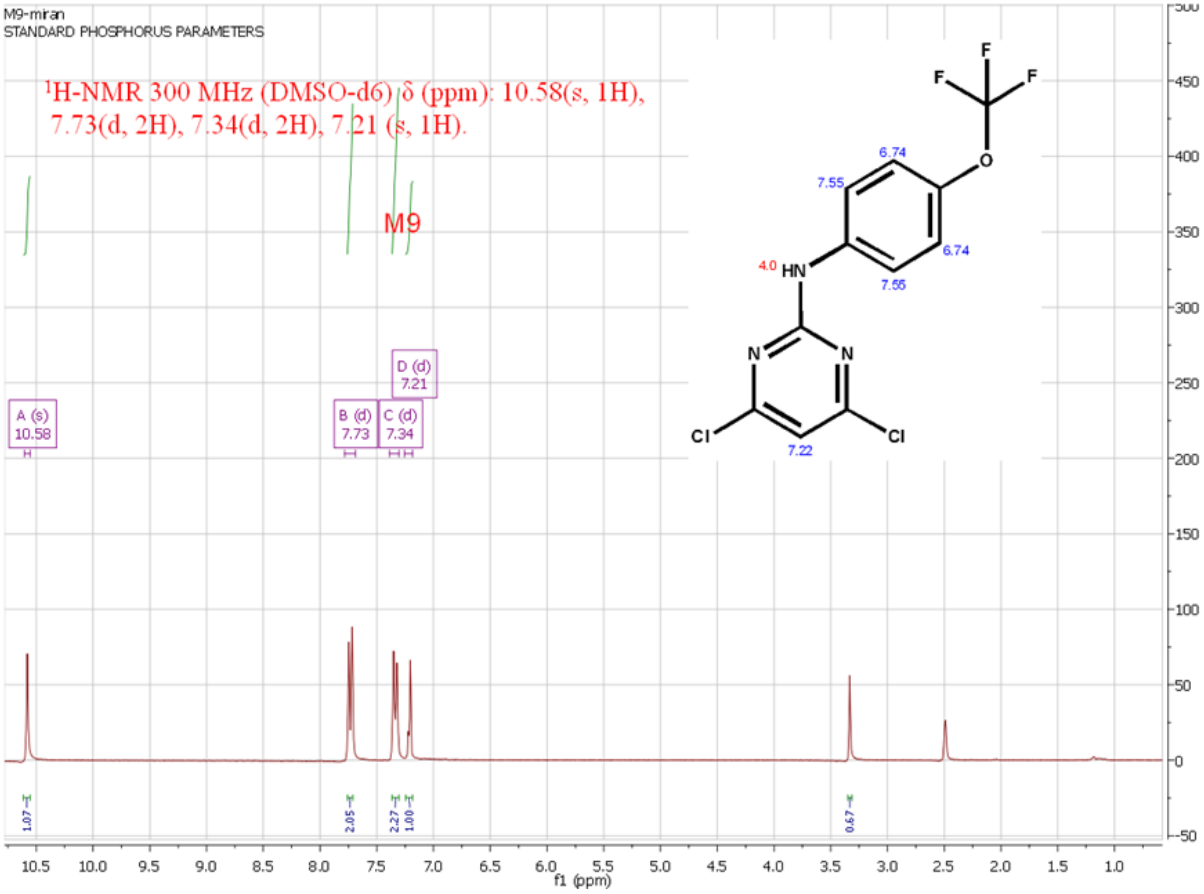

1h_tri_TFMA
DMSO
Gradient Shimmir
^{1}H-NMR 300 MHz (DMSO) δ (ppm): 10.41(s, 1H), 7.66(d, 2H), 7.40(d, 2H), 6.79 (s, 1H)
M3
H2O
DMSO
A (s) 10.41
B (d) 7.66
C (d) 7.40
D (s) 6.79
E (s) 3.35
f1 (ppm)

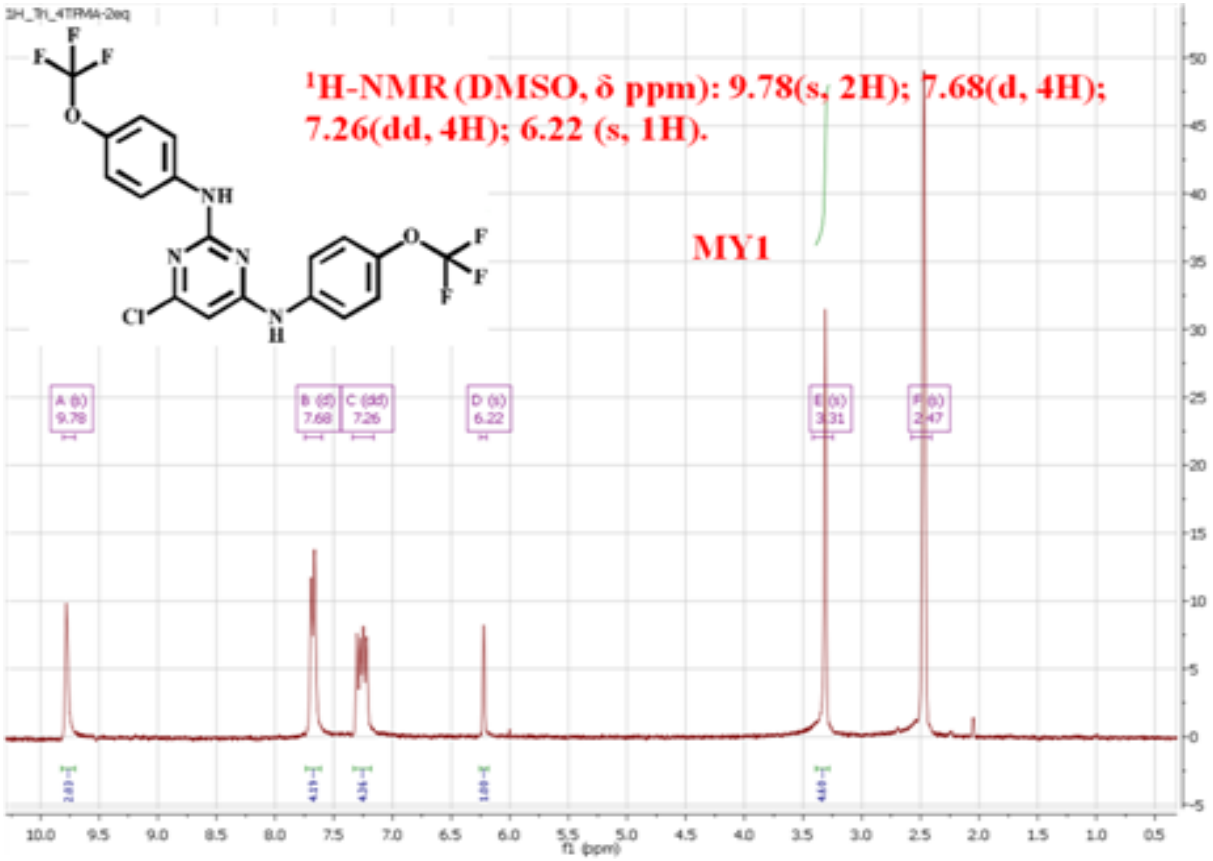

M4-miran
STANDARD PHOSPHORUS PARAMETERS
^{1}H-NMR 300 MHz (DMSO) δ (ppm): 8.4(s, 1H), 7.79(d, 1H), 7.40(d, 1H), 6.95 (s, 1H), 3.75 (t, 8h)
M4
A (m) 8.40
B (m) 7.79
C (m) 7.04
D (m) 6.95
E (s) 3.75
F (m) 3.25
G (m) 2.44
f1 (ppm)

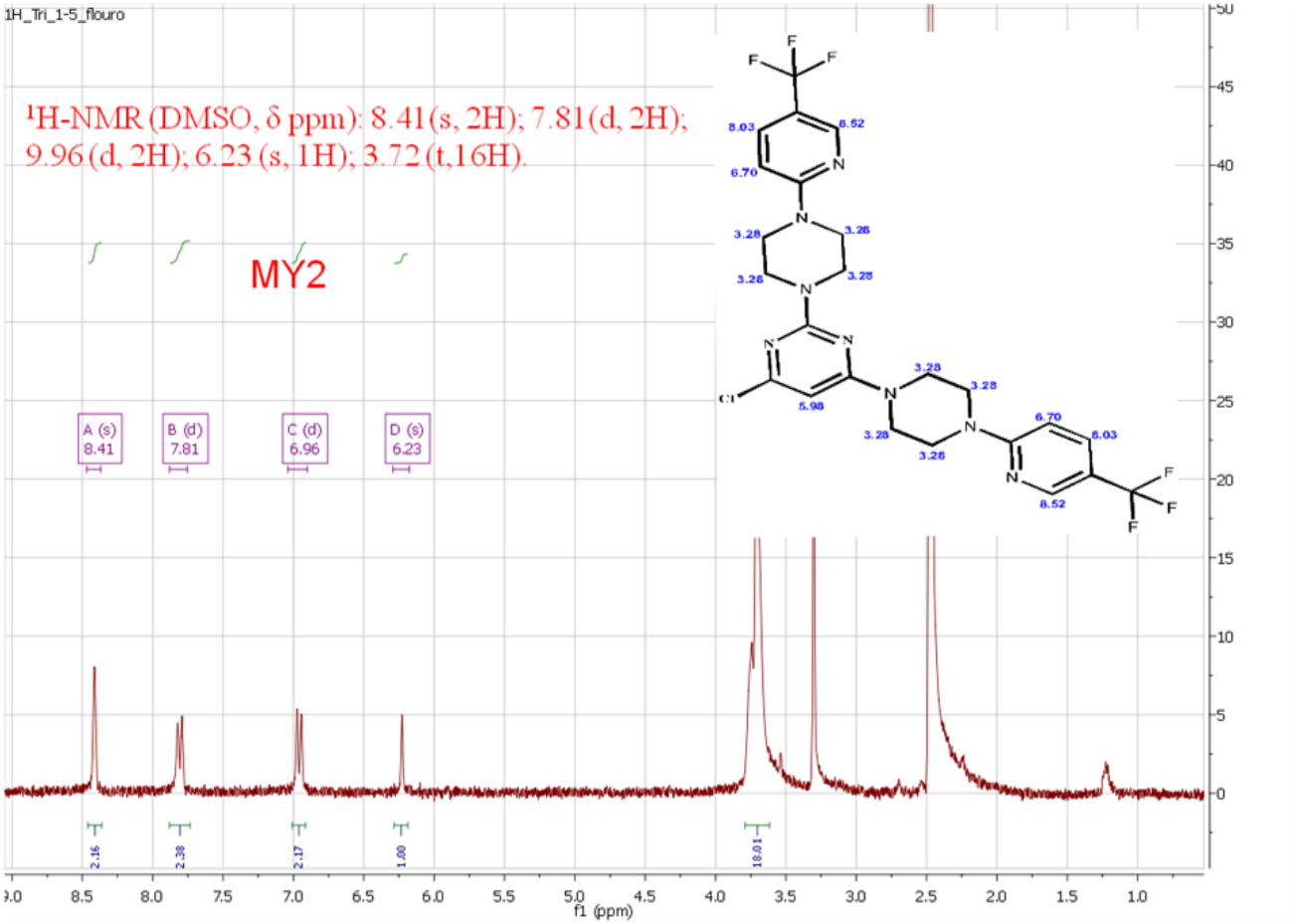

1H_Tri_1-3-P_position4
STANDARD FLUORINE PARAMETERS
8.53(d,1H),8.09(d,1H),7.21(t,1H),7.06 (s,1
3.77(t,4H),3.29(t,4H).
M5
Cl
N
N
Cl
6.46
3.28
3.28
N
3.28
N
3.28
F
F
F
7.32
N
6.38
8.07
B (s) 8.09
D (s) 7.06
F (d) 3.29
A (s) 8.53
C (s) 7.21
E (s) 3.77
G (s) 2.49
1.00
0.96
1.03
0.88
4.17
4.14
1.69

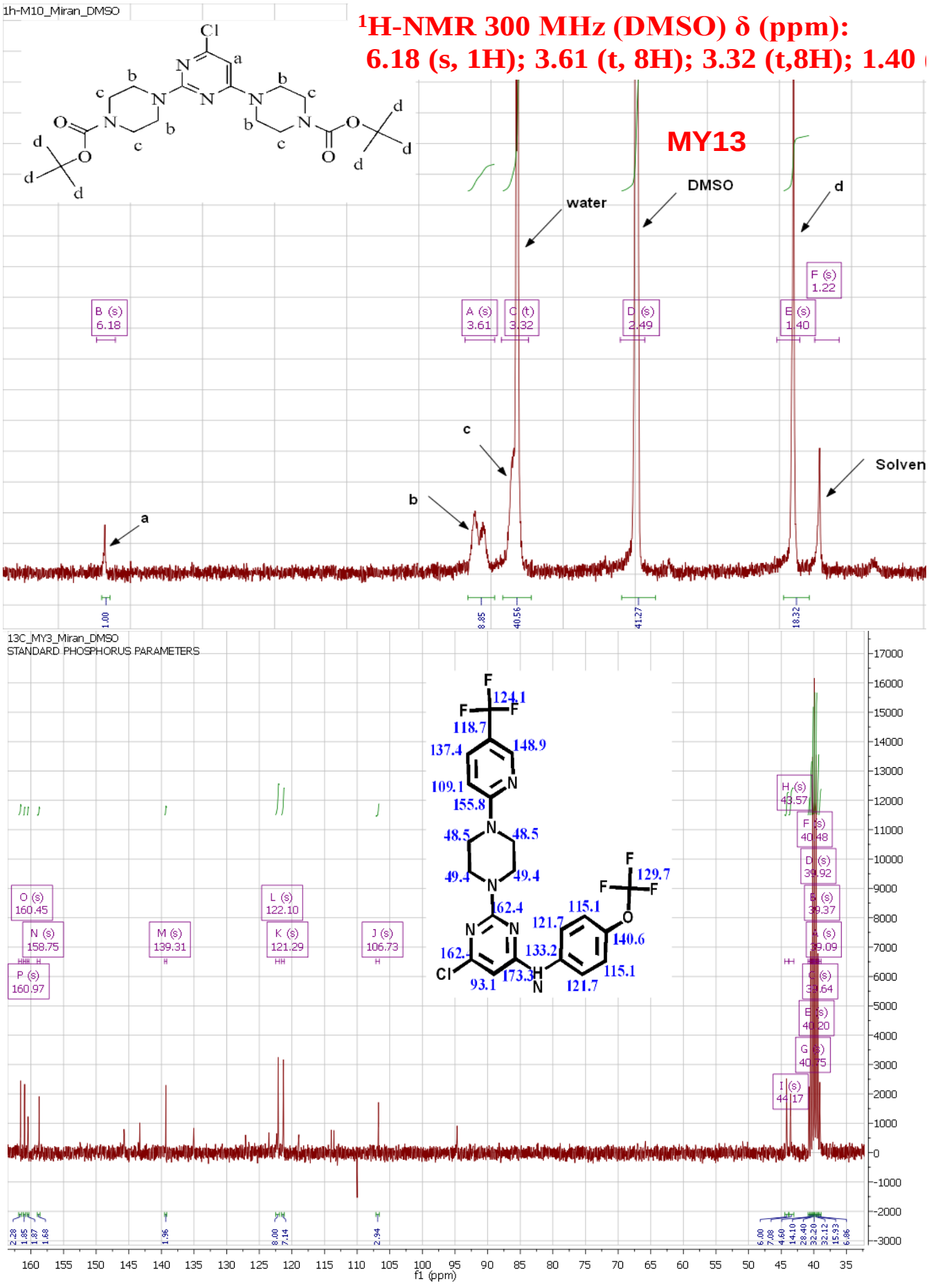

Trit-phipiprize
STANDARD PHOSPHORUS PARAMETERS
M6
1H-NMR 300 MHz (DMSO) δ (ppm):7.23(d, 2H), 7.09 (s,
1H), 6.96 (d, 2H), 6.81(s, 1H), 3.79(t,4H),3.21(t,4H)
Cl
N
N
Cl
2.92
3.21
N
2.92
3.21
N
6.94
7.27
6.94
6.79
7.27
D (t) 7.23
B (d) 6.96
C (s) 7.09
A (t) 6.81
E (s) 3.79
G (s) 3.21
F (s) 3.34
1.95
0.97
2.03
1.00
3.80
1.18
4.14
f1 (ppm)


1H_Miran_X
STANDARD PHOSPHORUS PARAMETERS
Tri+2-1pp
8.39 (d, 2H); 7.06 (s, 1H); 6.67(t,1H);3.78 (t,4H);3.32(t,4H)
M7
A (d) 8.39
B (s) 7.06
C (t) 6.67
D (d) 3.78
E (s) 3.32
F (s) 2.49
f1 (ppm)

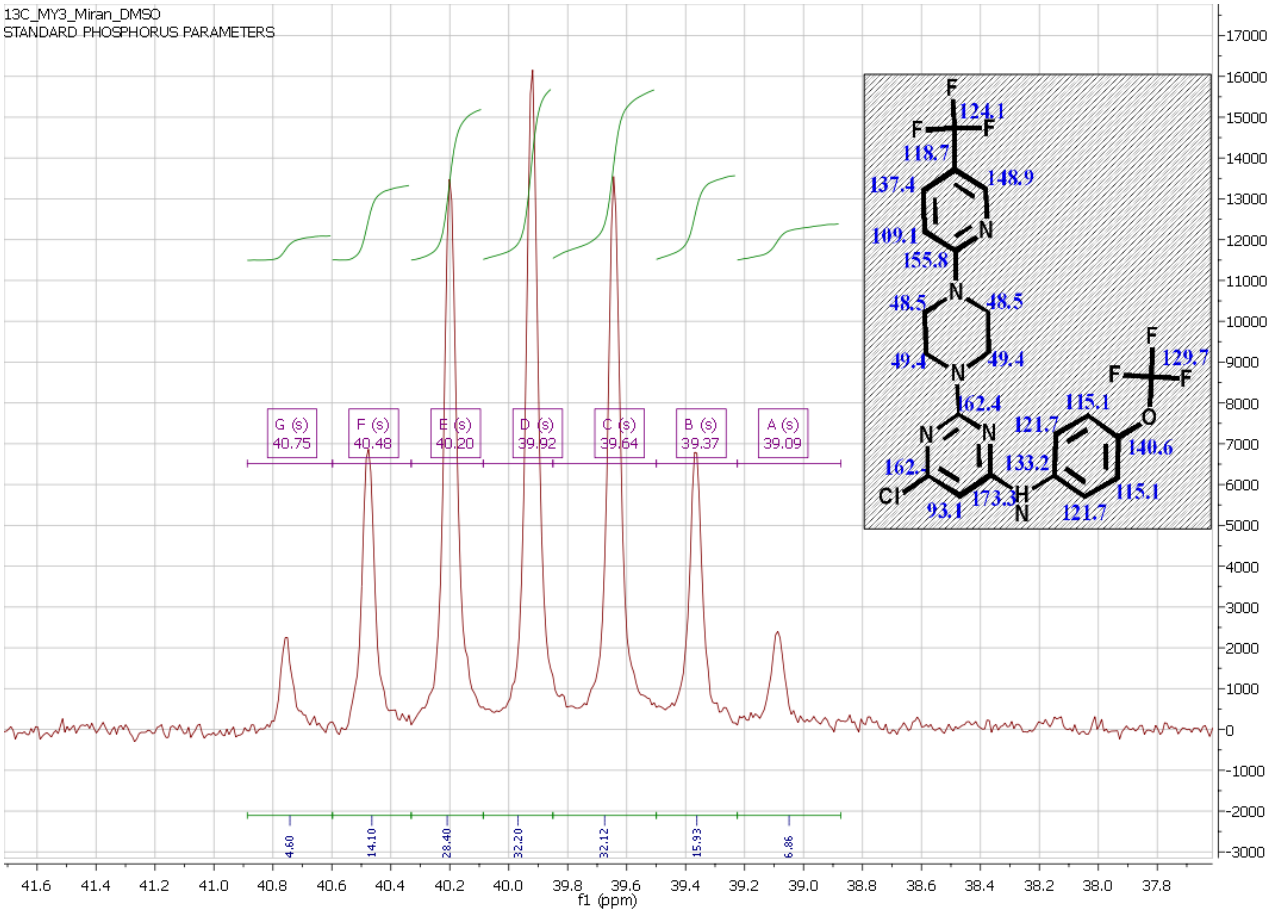

M9-miran
STANDARD PHOSPHORUS PARAMETERS
1H-NMR 300 MHz (DMSO-d6) δ (ppm): 10.58(s, 1H), 7.73(d, 2H), 7.34(d, 2H), 7.21 (s, 1H).
M9
A (s) 10.58
B (d) 7.73
C (d) 7.34
D (d) 7.21
f1 (ppm)

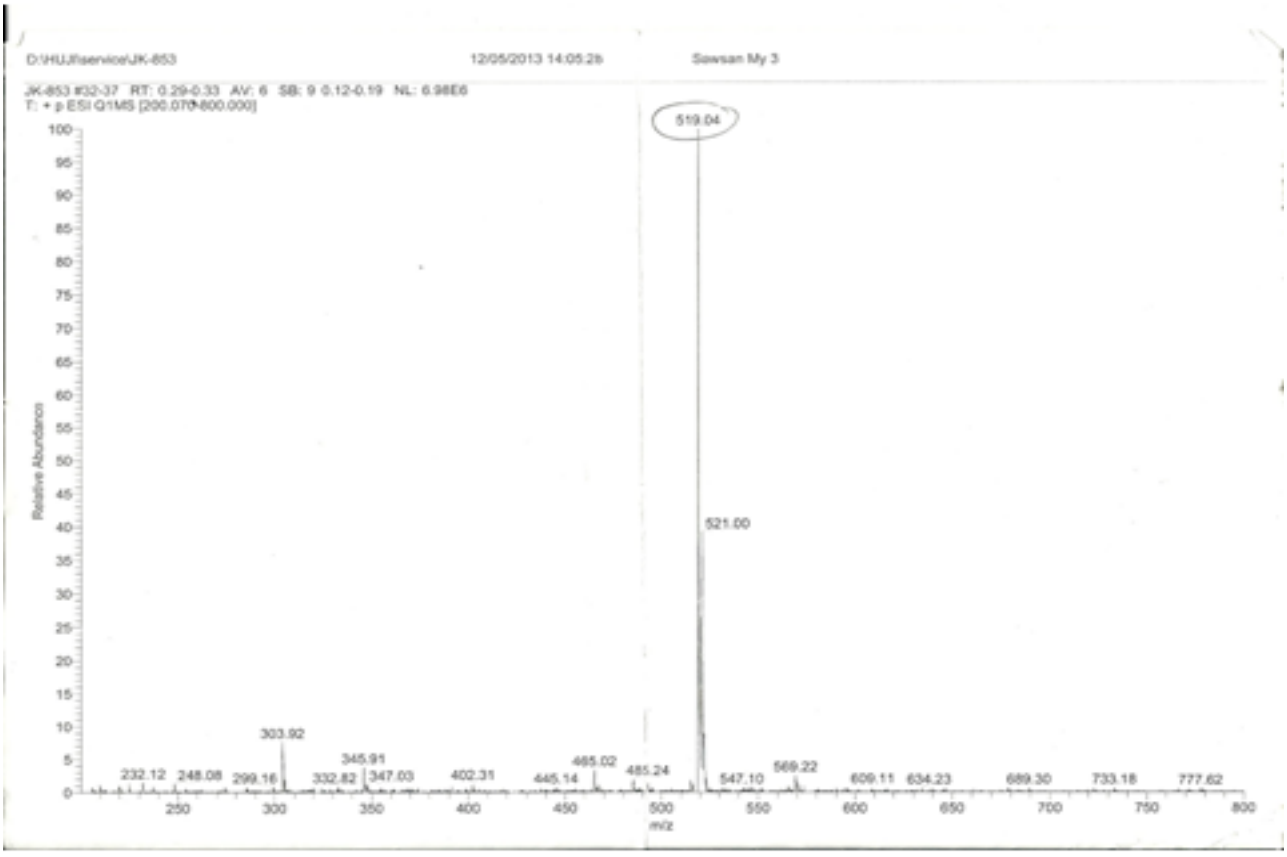

1H-NMR (DMSO, δ ppm): 9.78(s, 2H); 7.68(d, 4H); 7.26(dd, 4H); 6.22 (s, 1H).
MY1
f1 (ppm)


1H-NMR (DMSO, δ ppm): 8.41(s, 2H); 7.81(d, 2H); 9.96(d, 2H); 6.23 (s, 1H); 3.72 (t,16H).
MY2
f1 (ppm)



^{1}H-NMR 300 MHz (DMSO) δ (ppm):
6.18 (s, 1H); 3.61 (t, 8H); 3.32 (t,8H); 1.40 (


13C_MY3_Miran_DMSO
STANDARD PHOSPHORUS PARAMETERS
G (s) 40.75
F (s) 40.48
E (s) 40.20
D (s) 39.92
C (s) 39.64
B (s) 39.37
A (s) 39.09
4.60
14.10
28.40
32.20
32.12
15.93
6.86
f1 (ppm)

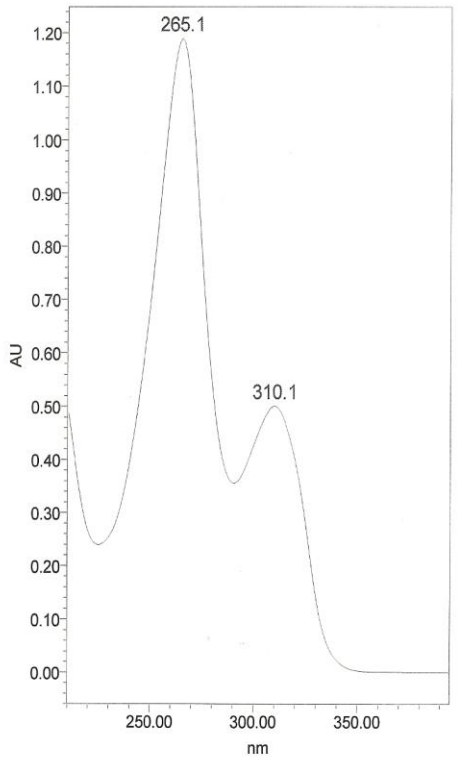

519.04
521.00
303.92
345.91
Relative Abundance
m/z

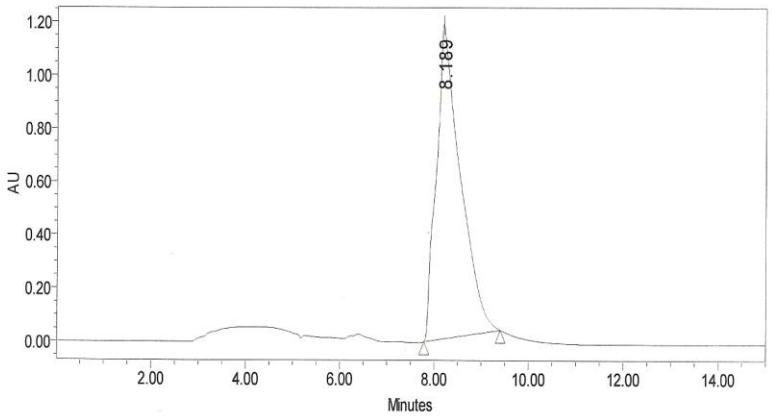
St. Phase : C18 , 250X4.6 mm columr

Mob. Phase : ACN : Water , 97 : 3

Flow rate :1 ml / min

volume injection : 10 µl





	Processed Channel Descr.	RT	Area	% Area	Height
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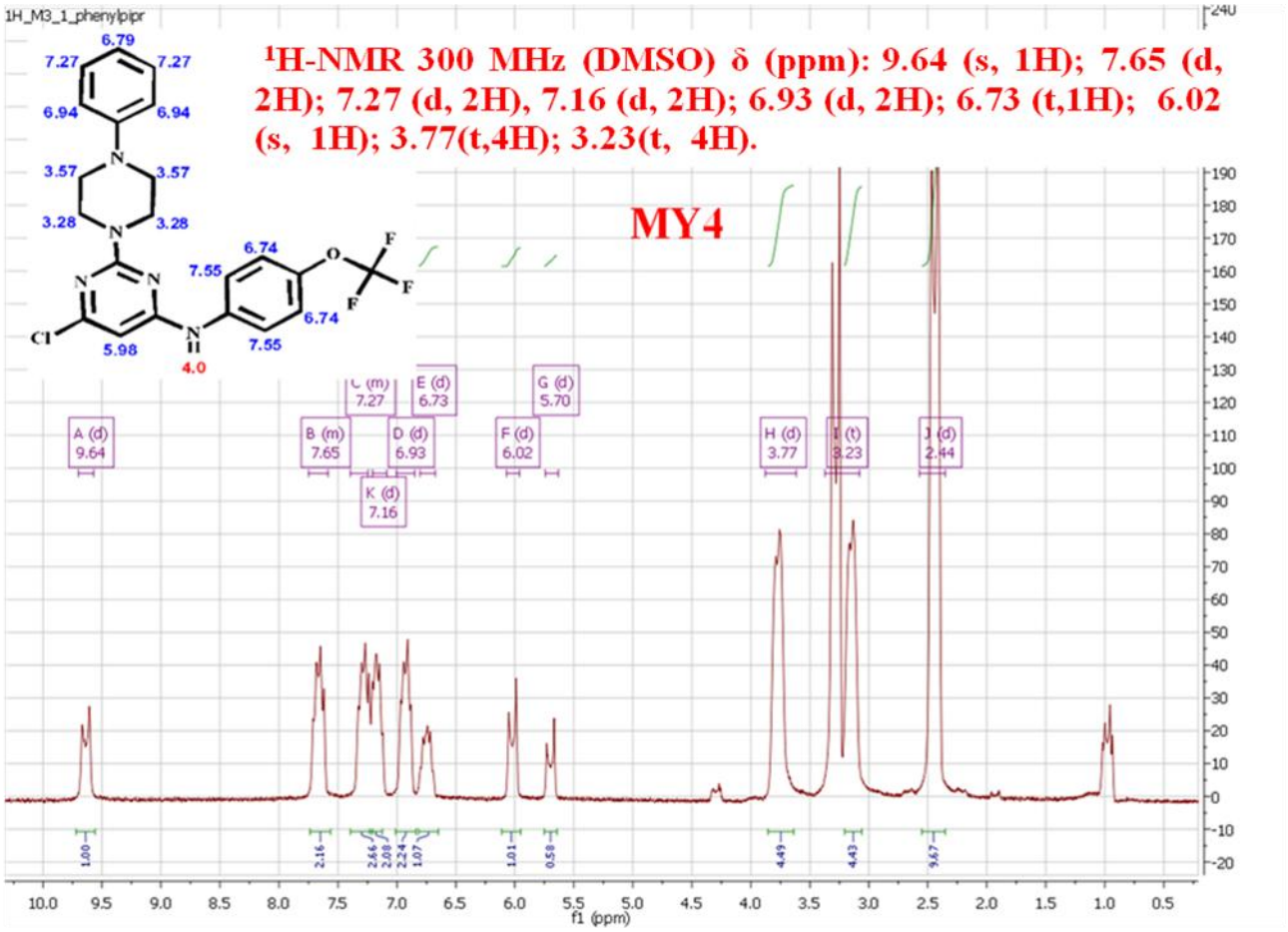
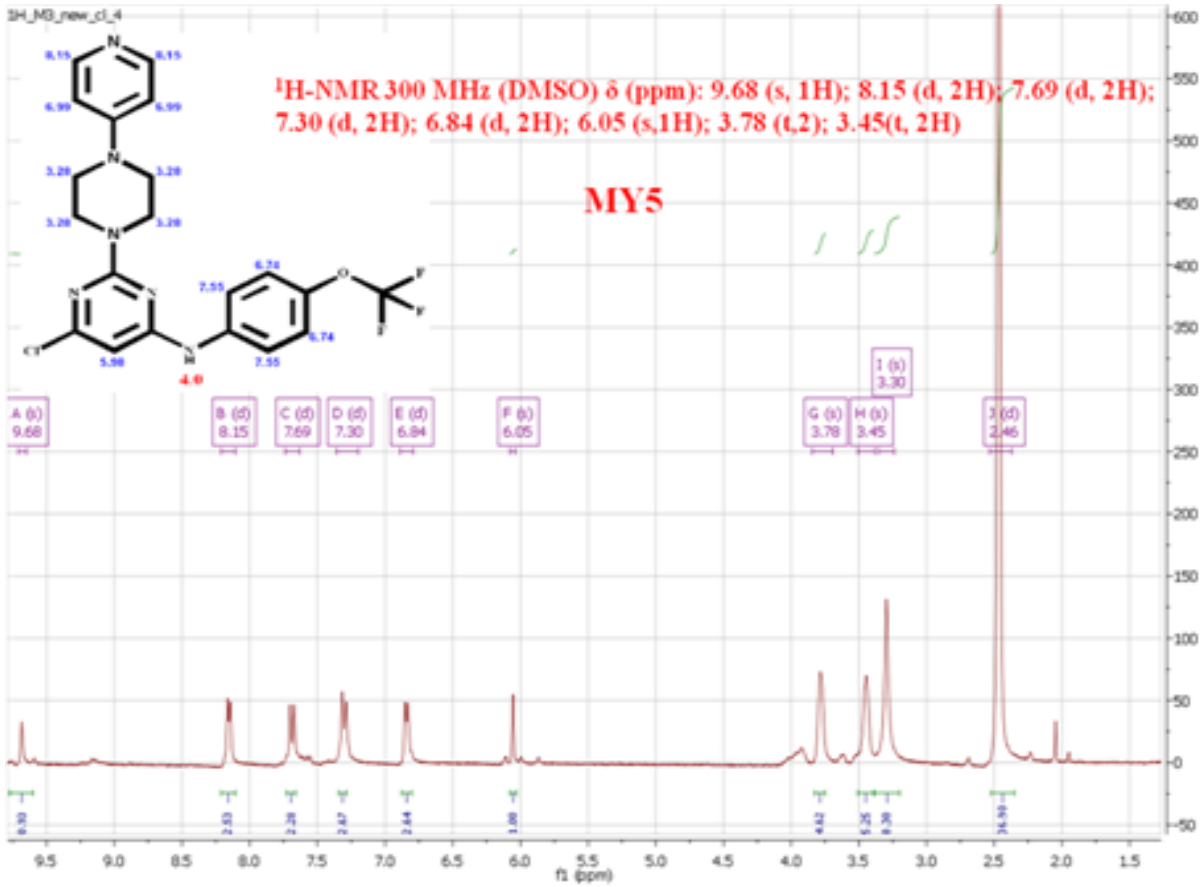
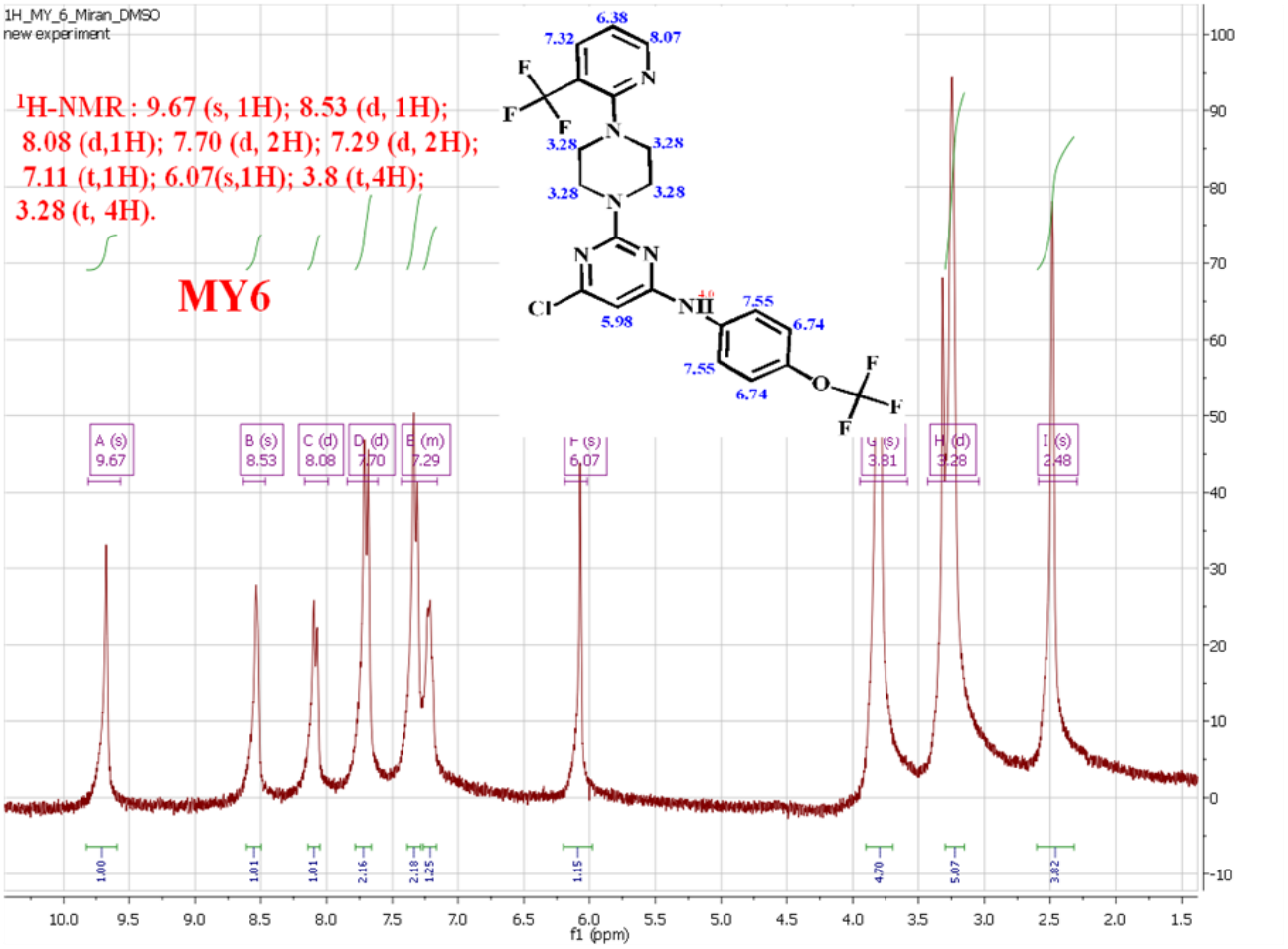
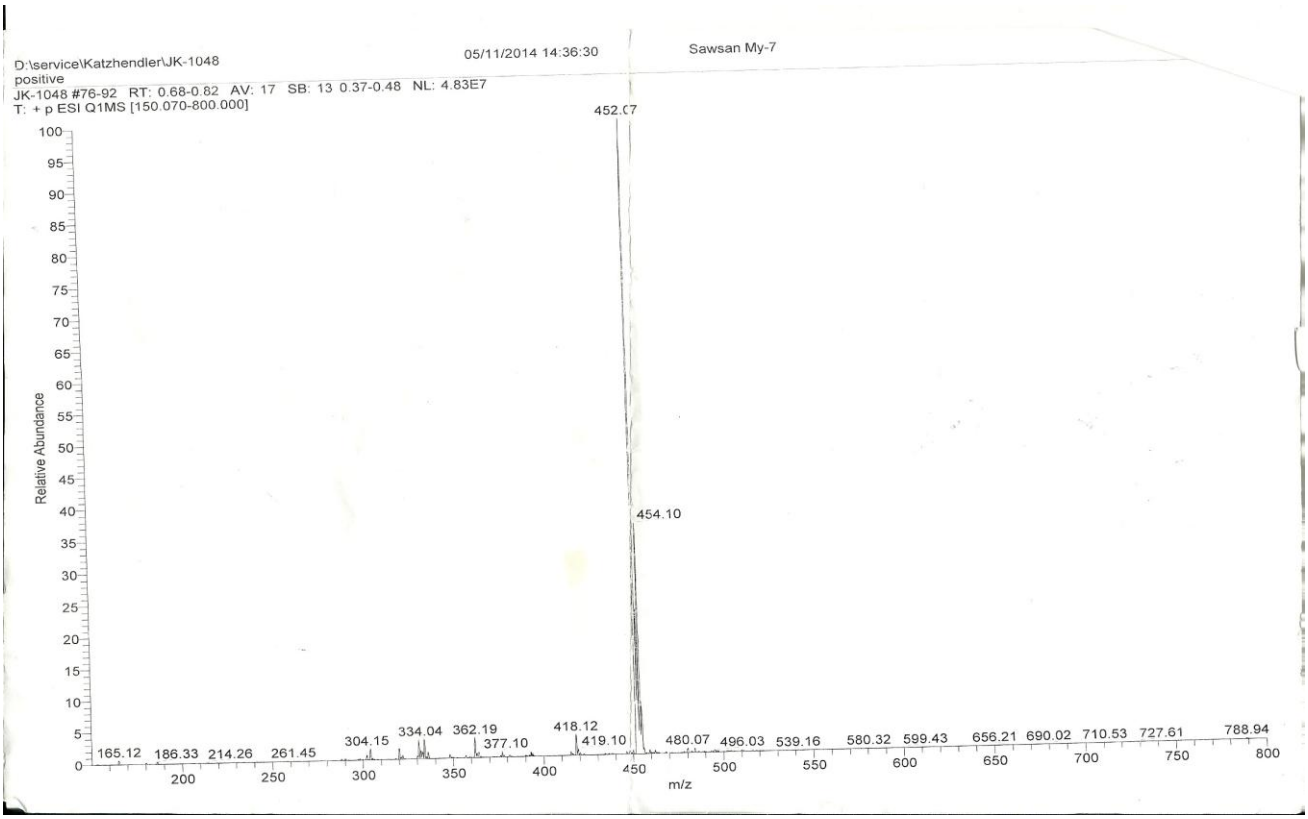
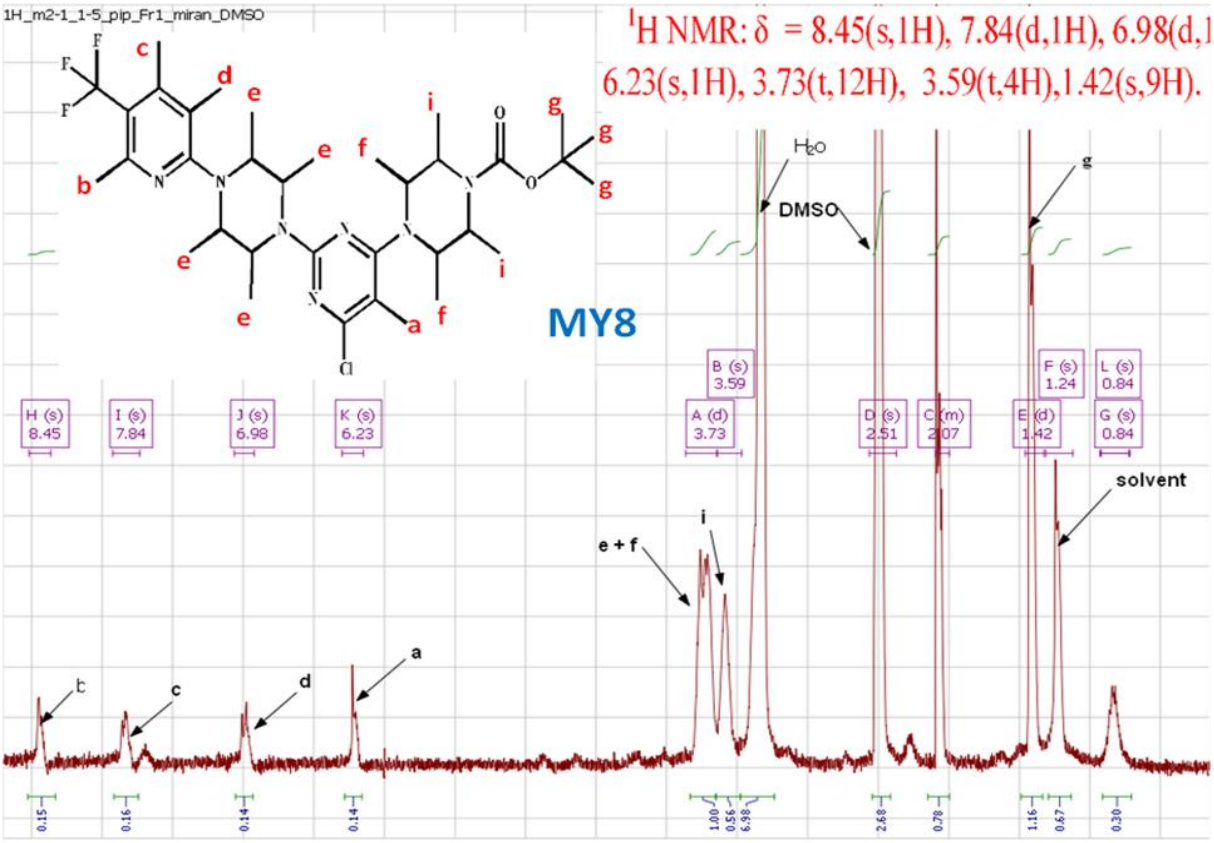
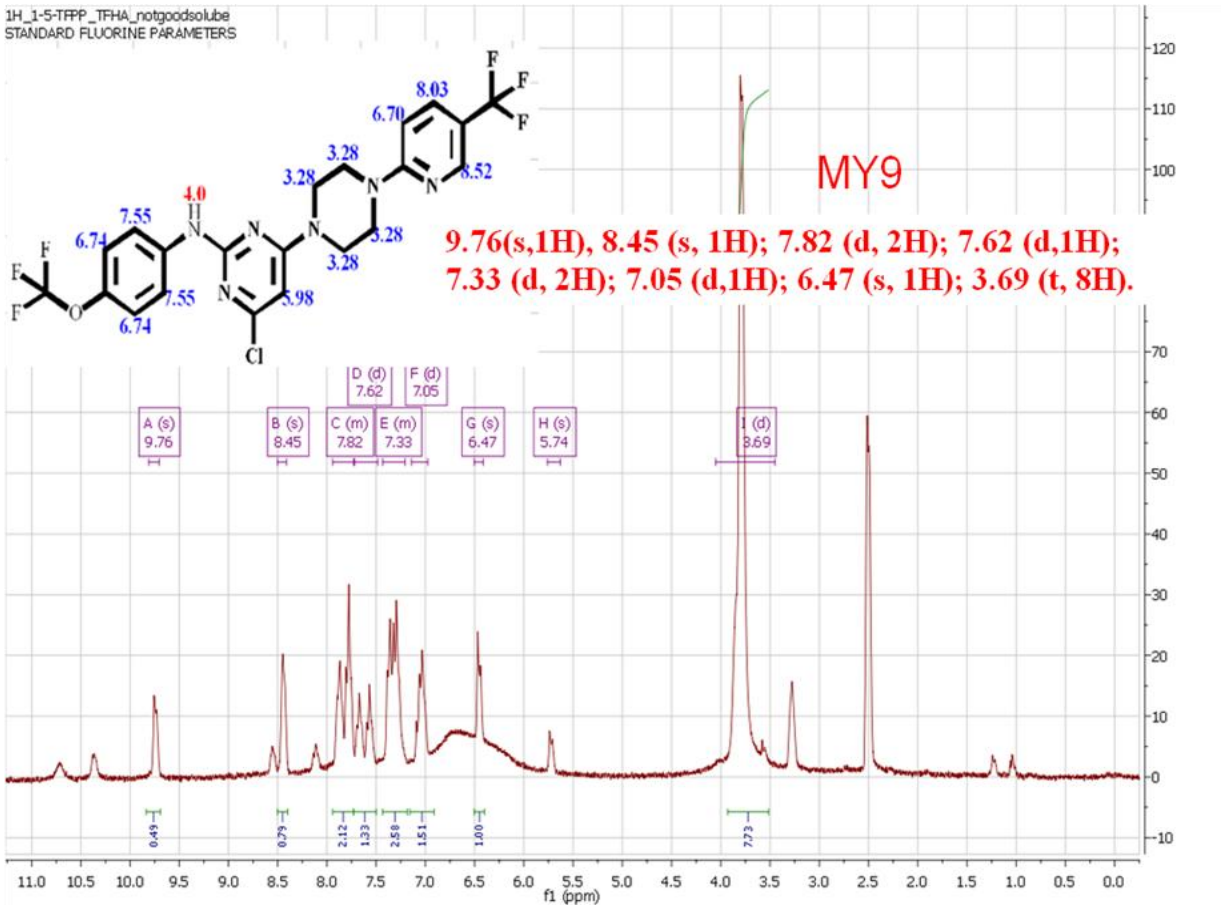

1H_M3_1_phenylpipr
¹H-NMR 300 MHz (DMSO) δ (ppm): 9.64 (s, 1H); 7.65 (d, 2H); 7.27 (d, 2H), 7.16 (d, 2H); 6.93 (d, 2H); 6.73 (t,1H); 6.02 (s, 1H); 3.77(t,4H); 3.23(t, 4H).
MY4
f1 (ppm)

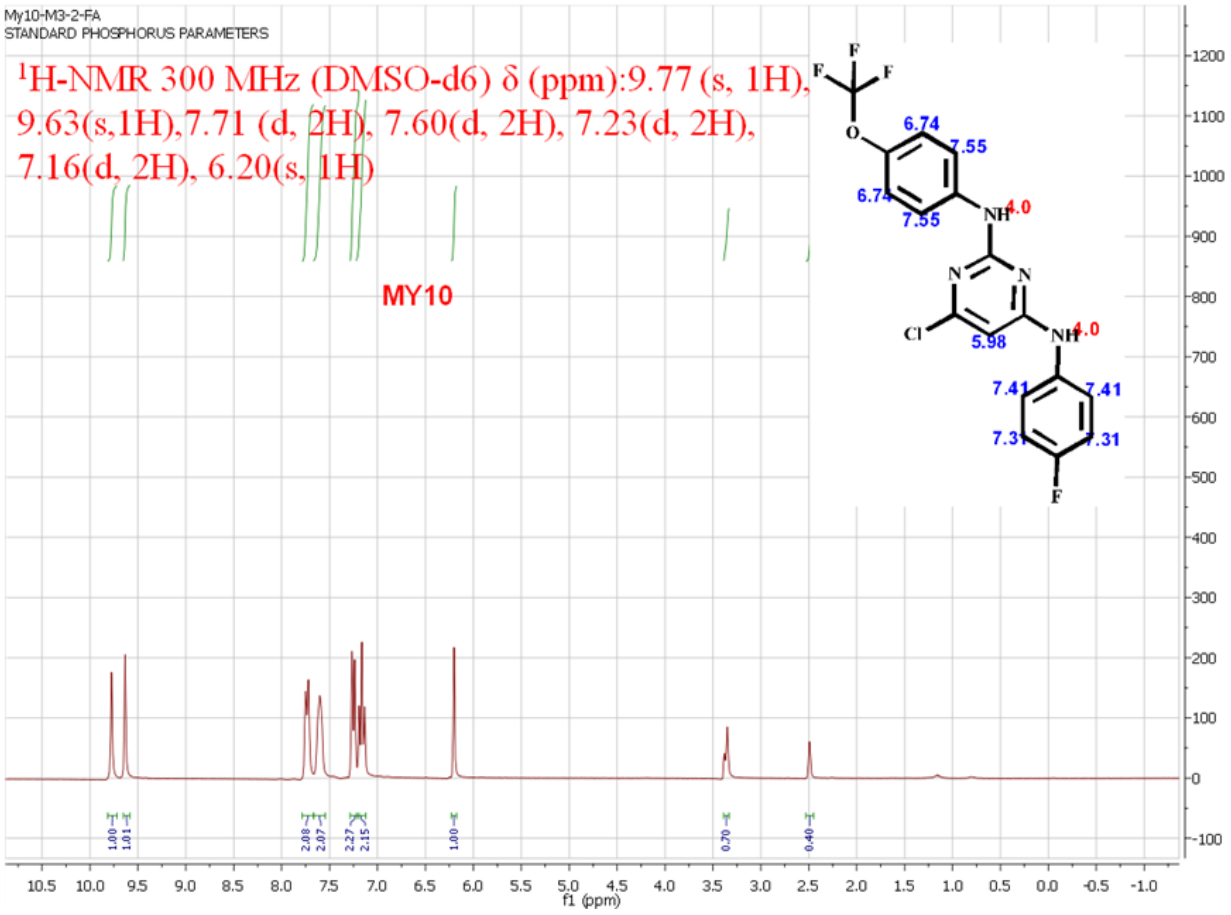

¹H-NMR 300 MHz (DMSO) δ (ppm): 9.68 (s, 1H); 8.15 (d, 2H); 7.69 (d, 2H); 7.30 (d, 2H); 6.84 (d, 2H); 6.05 (s,1H); 3.78 (t,2); 3.45(t, 2H)
MY5
f1 (ppm)

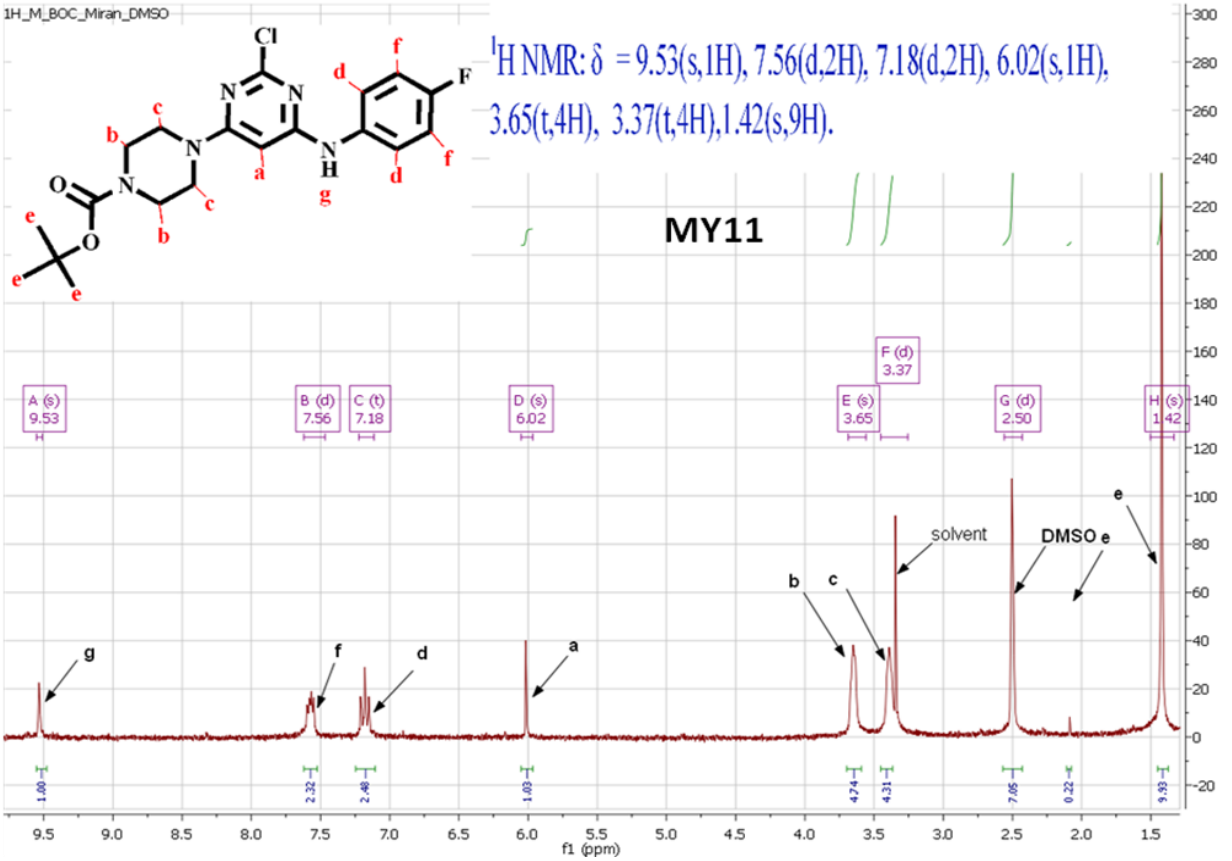

1H_MY_6_Miran_DMSO
new experiment
1H-NMR : 9.67 (s, 1H); 8.53 (d, 1H);
8.08 (d,1H); 7.70 (d, 2H); 7.29 (d, 2H);
7.11 (t,1H); 6.07(s,1H); 3.8 (t,4H);
3.28 (t, 4H).
MY6
A (s) 9.67
B (s) 8.53
C (d) 8.08
D (d) 7.70
E (m) 7.29
F (s) 6.07
G (s) 3.81
H (d) 3.28
I (s) 2.48
f1 (ppm)

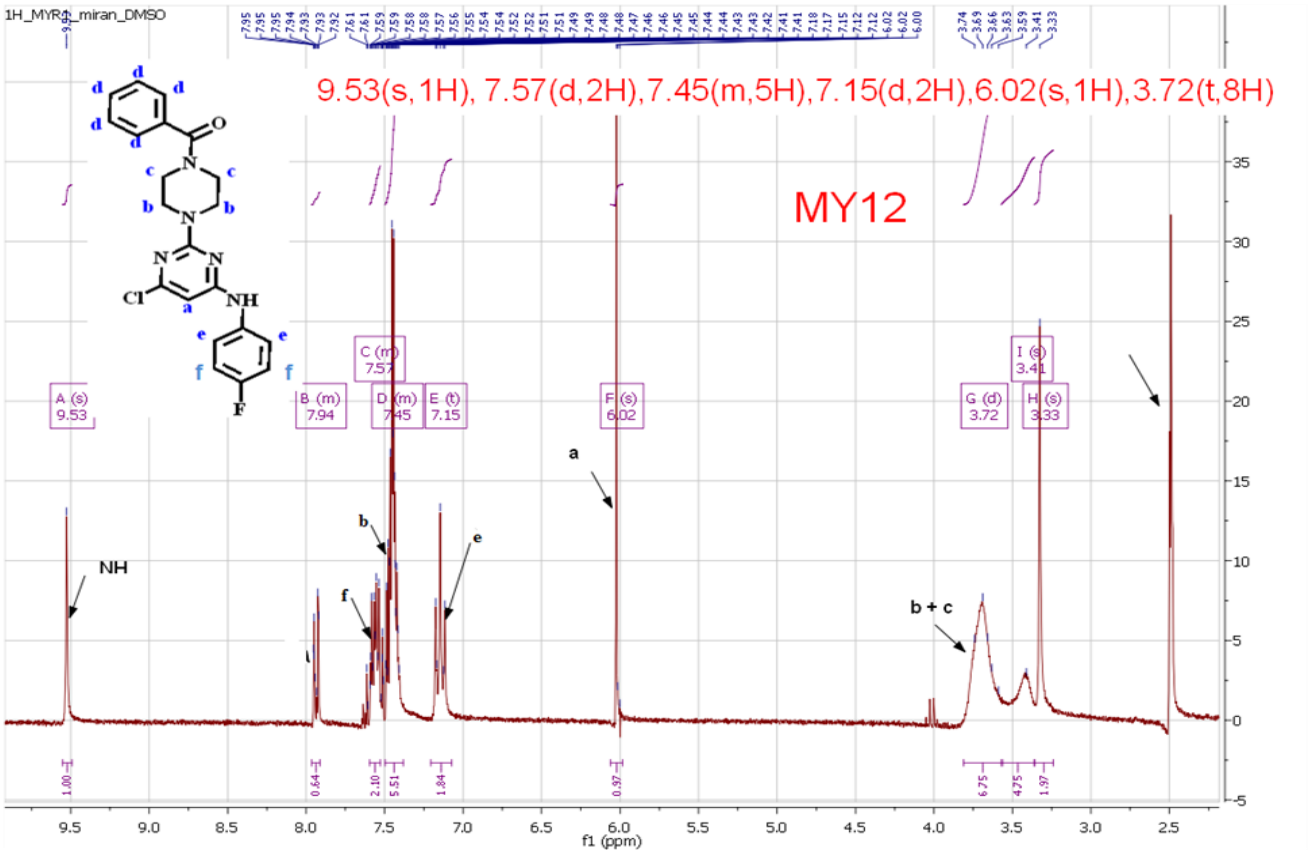

D:\service\Katzhendler\JK-1048
positive
JK-1048 #76-92 RT: 0.68-0.82 AV: 17 SB: 13 0.37-0.48 NL: 4.83E7
T: + p ESI Q1MS [150.070-800.000]
05/11/2014 14:36:30
Sawsan My-7
452.07
454.10
Relative Abundance
m/z

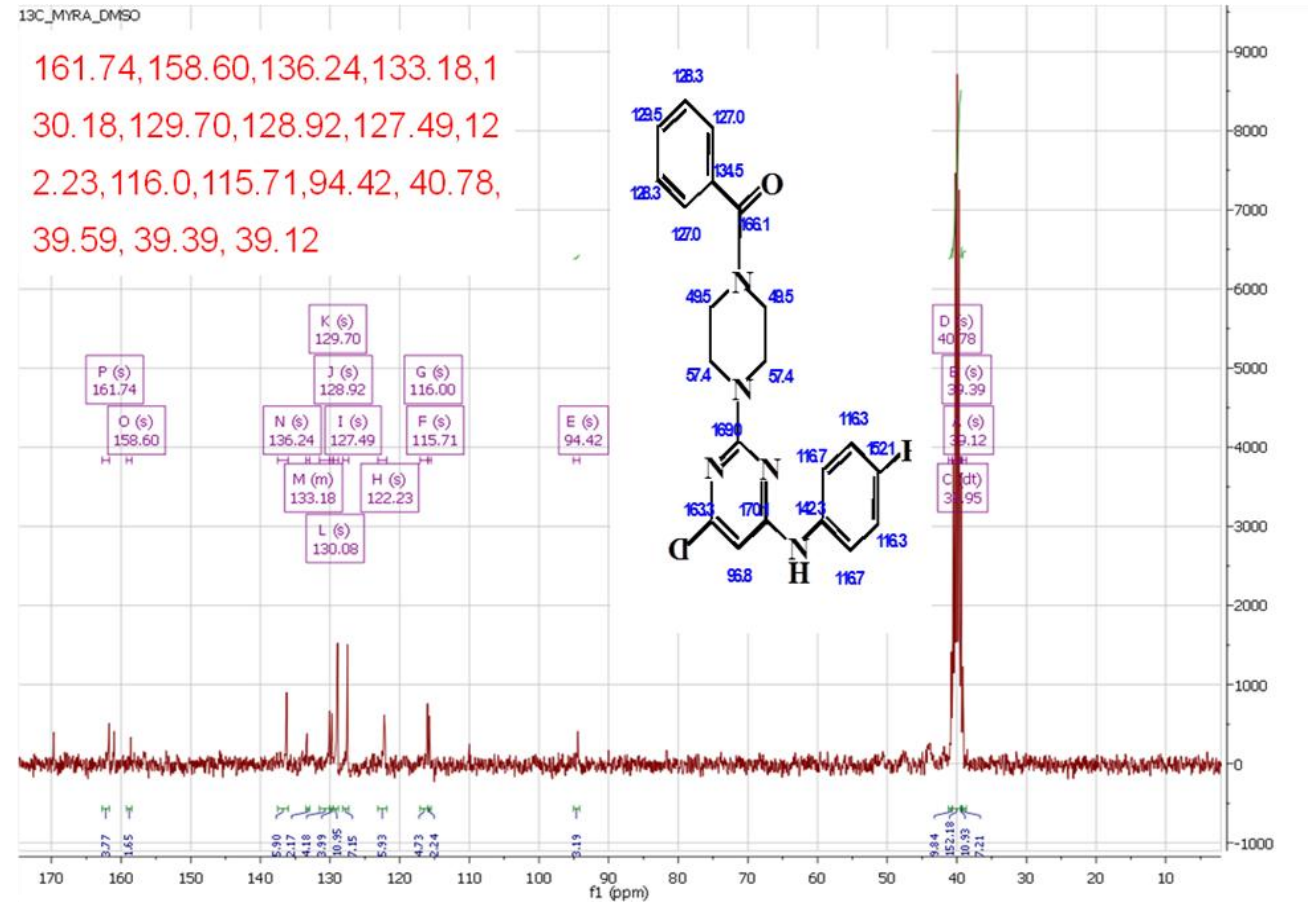

1H_m2-1_1-5_pip_Fr1_miran_DMSO
^{1}H NMR: δ = 8.45(s,1H), 7.84(d,1H), 6.98(d,1
6.23(s,1H), 3.73(t,12H), 3.59(t,4H),1.42(s,9H).
MY8
H2O
DMSO
solvent
e + f
i
a
b
c
d
g
H (s) 8.45
I (s) 7.84
J (s) 6.98
K (s) 6.23
B (s) 3.59
A (d) 3.73
D (s) 2.51
C (m) 2.07
E (d) 1.42
F (s) 1.24
G (s) 0.84
L (s) 0.84

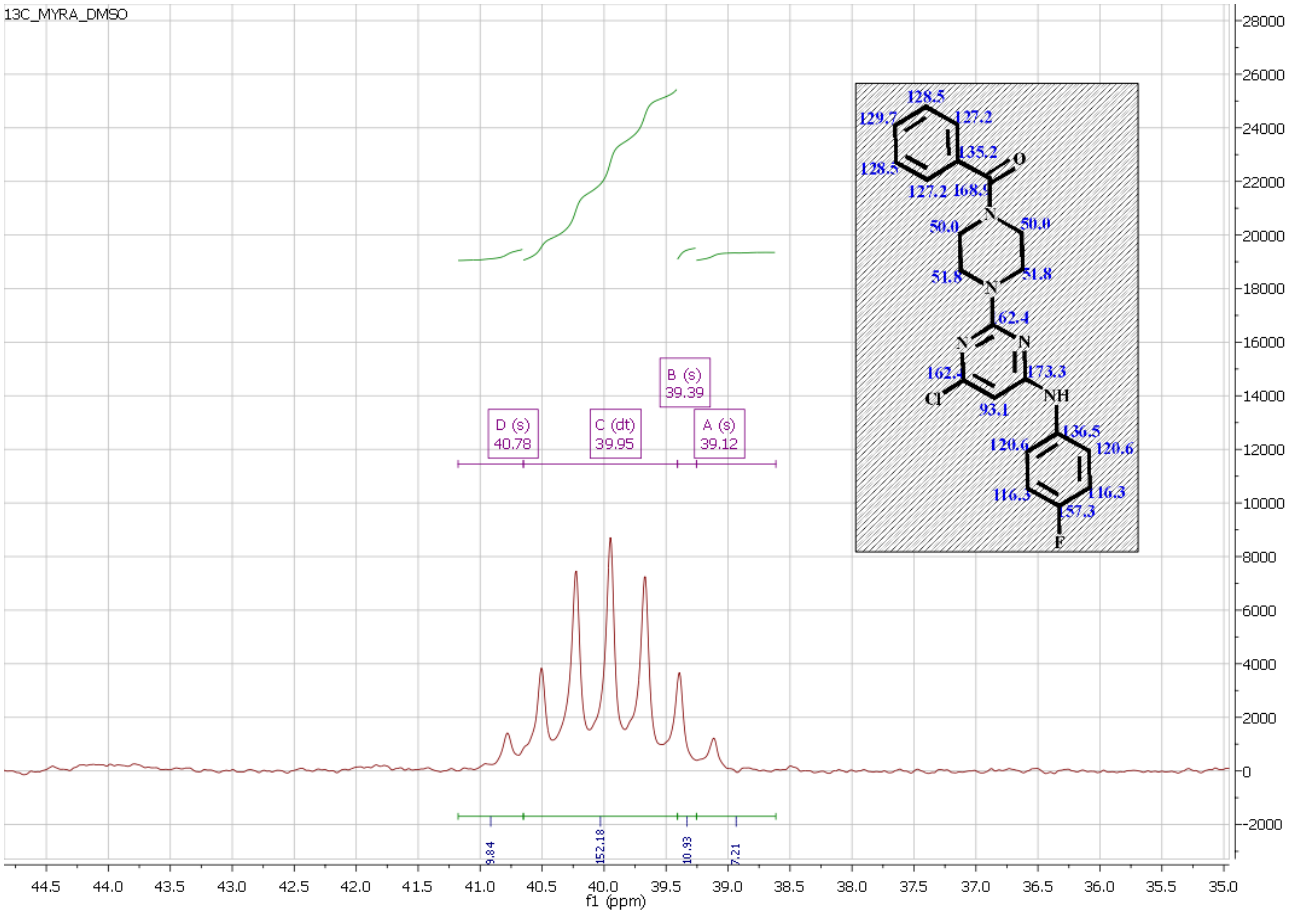

1H_1-5-TFPP_TFHA_notgoodsolube
STANDARD FLUORINE PARAMETERS
MY9
9.76(s,1H), 8.45 (s, 1H); 7.82 (d, 2H); 7.62 (d,1H);
7.33 (d, 2H); 7.05 (d,1H); 6.47 (s, 1H); 3.69 (t, 8H).
8.03
6.70
3.28
3.28
8.52
4.0
7.55
6.74
3.28
3.28
7.55
5.98
6.74
Cl
A (s) 9.76
B (s) 8.45
C (m) 7.82
D (d) 7.62
E (m) 7.33
F (d) 7.05
G (s) 6.47
H (s) 5.74
I (d) 3.69
f1 (ppm)

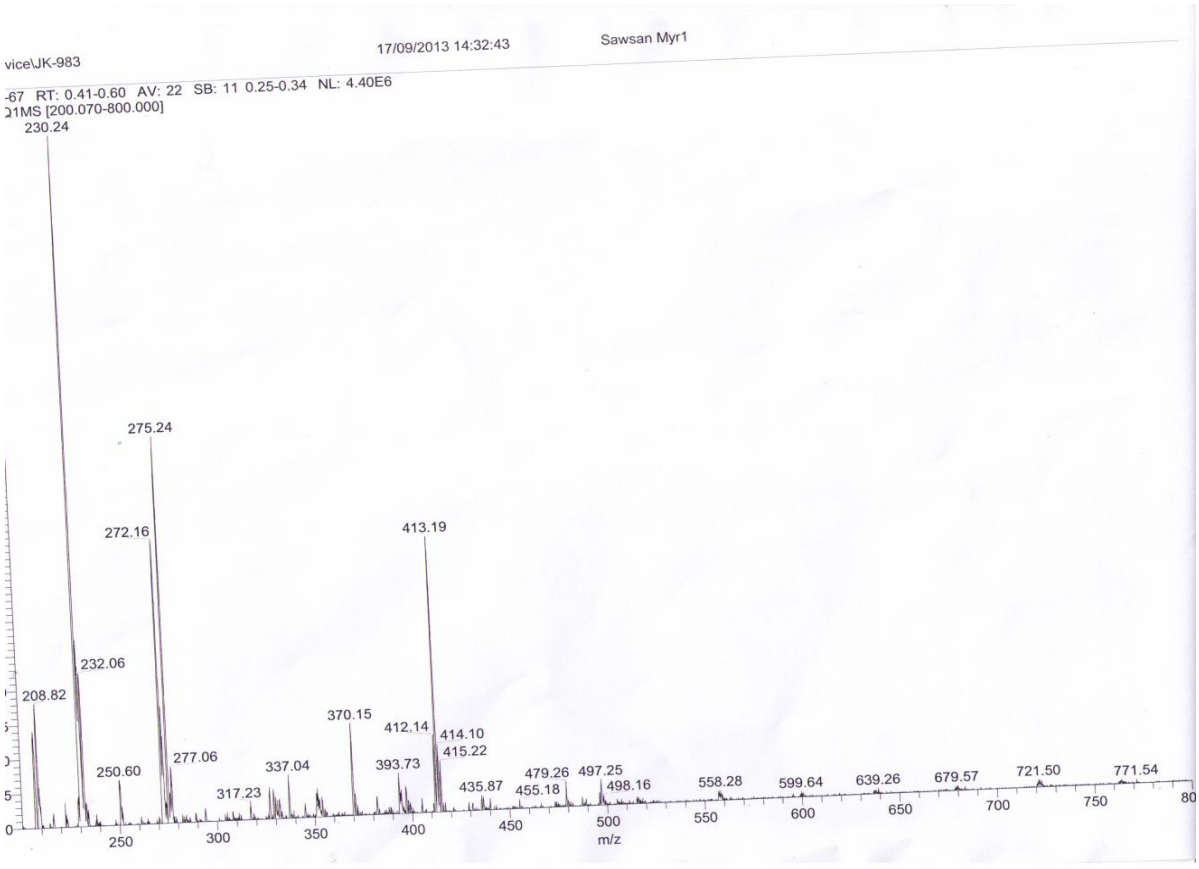

My10-M3-2-FA
STANDARD PHOSPHORUS PARAMETERS
¹H-NMR 300 MHz (DMSO-d6) δ (ppm):9.77 (s, 1H), 9.63(s,1H),7.71 (d, 2H), 7.60(d, 2H), 7.23(d, 2H), 7.16(d, 2H), 6.20(s, 1H)
MY10
f1 (ppm)

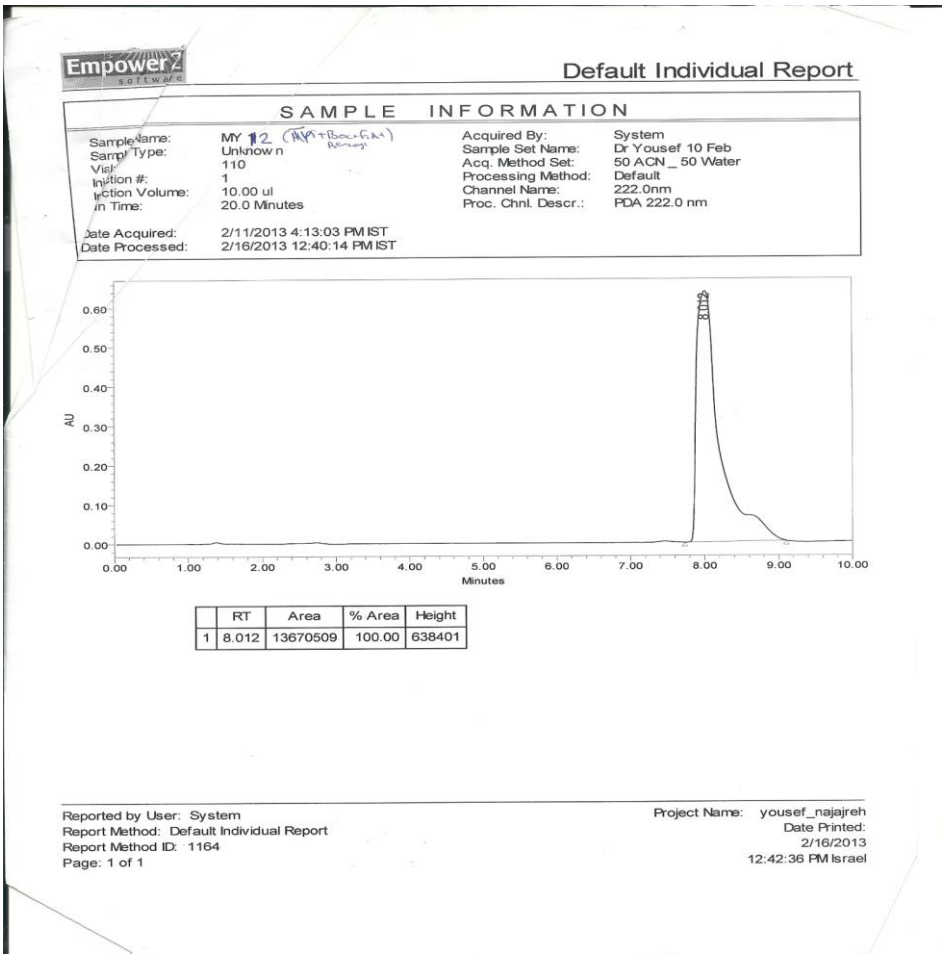

1H_M_BOC_Miran_DMSO
¹H NMR: δ = 9.53(s,1H), 7.56(d,2H), 7.18(d,2H), 6.02(s,1H), 3.65(t,4H), 3.37(t,4H),1.42(s,9H).
MY11
solvent
DMSO
f1 (ppm)


1H_MYRA_miran_DMSO
9.53(s,1H), 7.57(d,2H),7.45(m,5H),7.15(d,2H),6.02(s,1H),3.72(t,8H)
MY12
NH
a
b
e
f
b + c
f1 (ppm)


13C_MYRA_DMSO
161.74,158.60,136.24,133.18,1
30.18,129.70,128.92,127.49,12
2.23,116.0,115.71,94.42, 40.78,
39.59, 39.39, 39.12
f1 (ppm)


13C_MYRA_DMSO
B (s)
39.39
D (s)
40.78
C (dt)
39.95
A (s)
39.12
128.5
129.7
127.2
135.2
128.5
127.2
168.5
50.0
50.0
51.8
51.8
62.4
162.4
173.3
Cl
NH
93.1
136.5
120.6
120.6
116.3
116.3
157.3
F
f1 (ppm)


vice\JK-983
17/09/2013 14:32:43
Sawsan Myr1
-67 RT: 0.41-0.60 AV: 22 SB: 11 0.25-0.34 NL: 4.40E6
Q1MS [200.070-800.000]
230.24
275.24
272.16
413.19
232.06
208.82
370.15
412.14
414.10
415.22
277.06
250.60
337.04
393.73
317.23
435.87
455.18
479.26
497.25
498.16
558.28
599.64
639.26
679.57
721.50
771.54
m/z



Empower 2 software

Default Individual Report

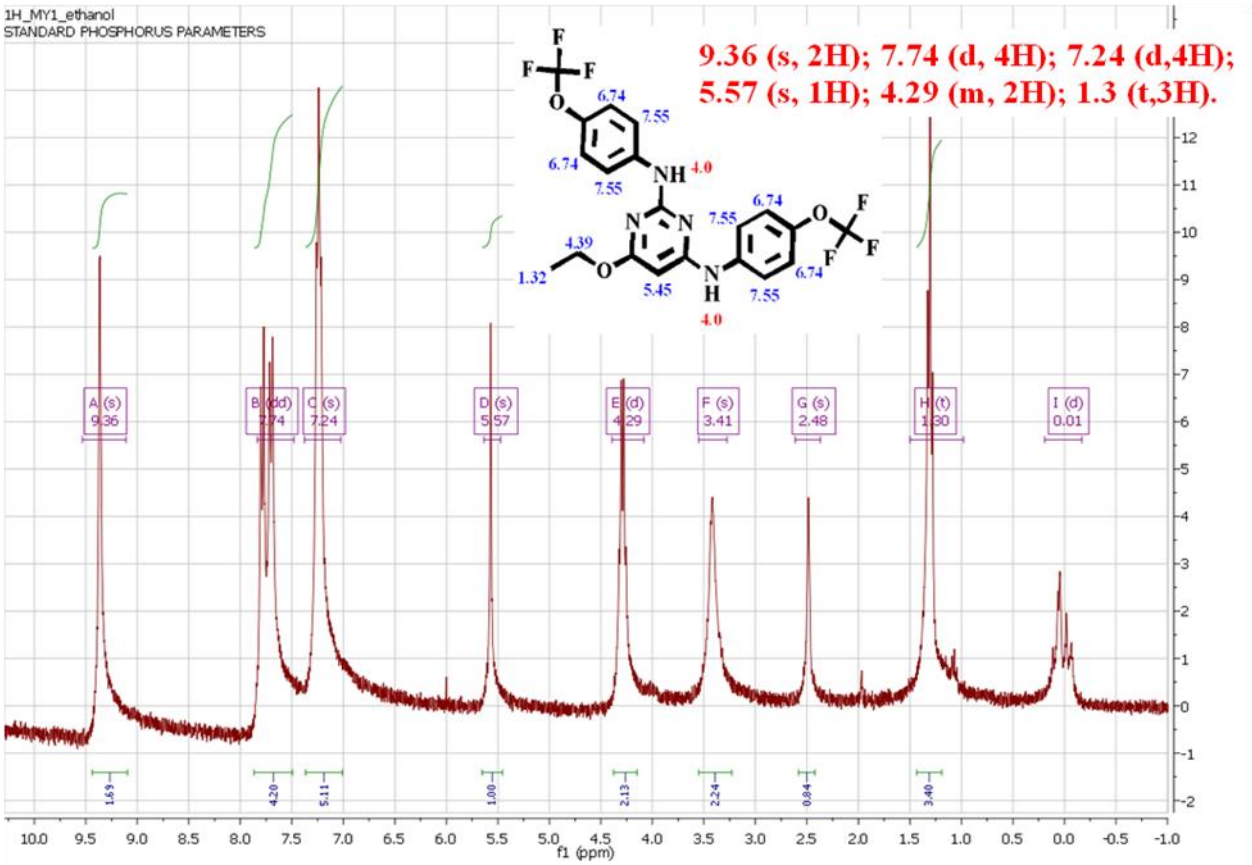
SAMPLE INFORMATION

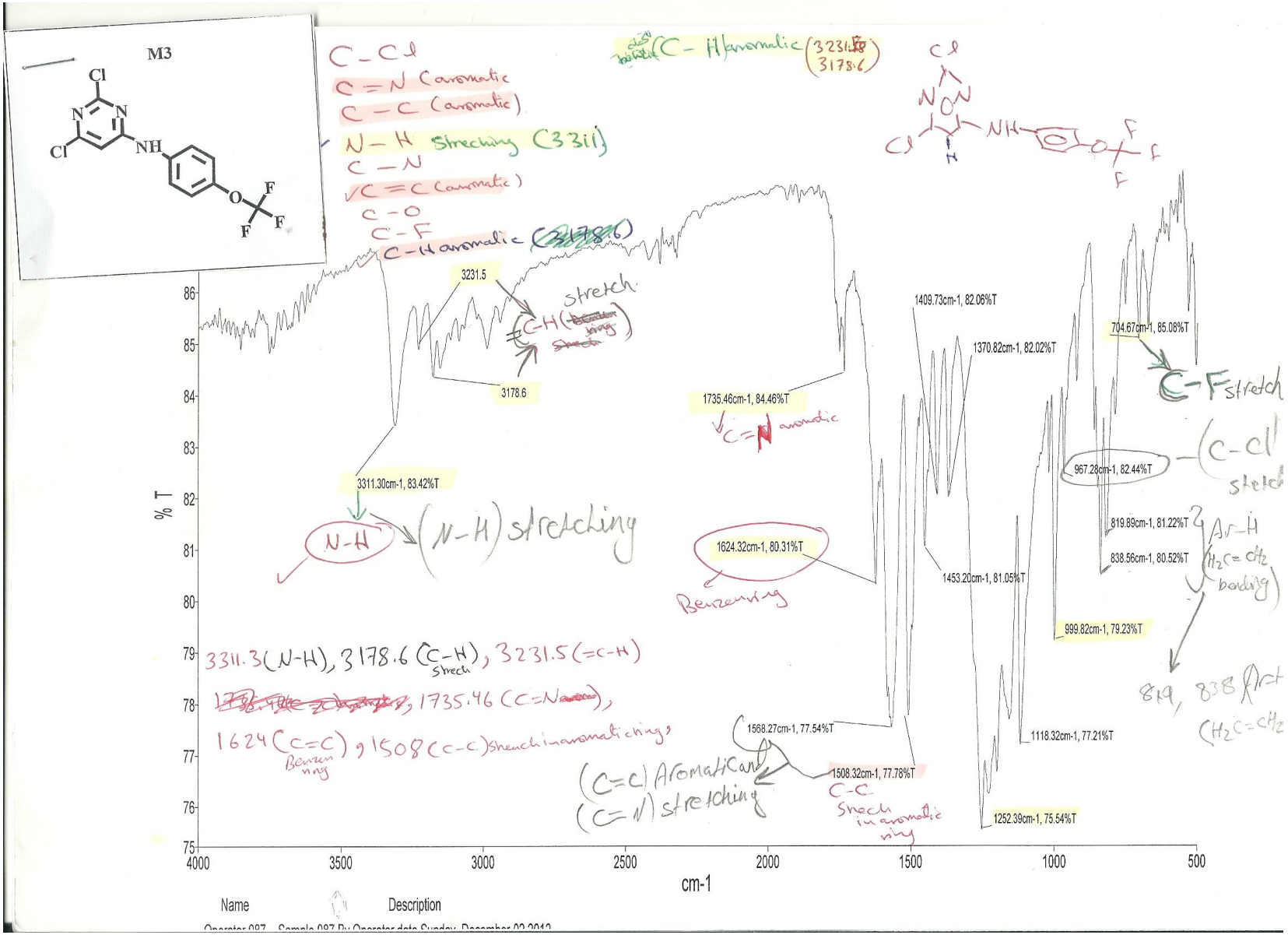
Sample Name:	MY 12 (BAY+Boric Acid Recrys)	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Dr Yousef 10 Feb
Vial:	110	Acq. Method Set:	50 ACN _ 50 Water
Injection #:	1	Processing Method:	Default
Injection Volume:	10.00 ul	Channel Name:	222.0nm
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	PDA 222.0 nm
Date Acquired:	2/11/2013 4:13:03 PM IST		
Date Processed:	2/16/2013 12:40:14 PM IST		

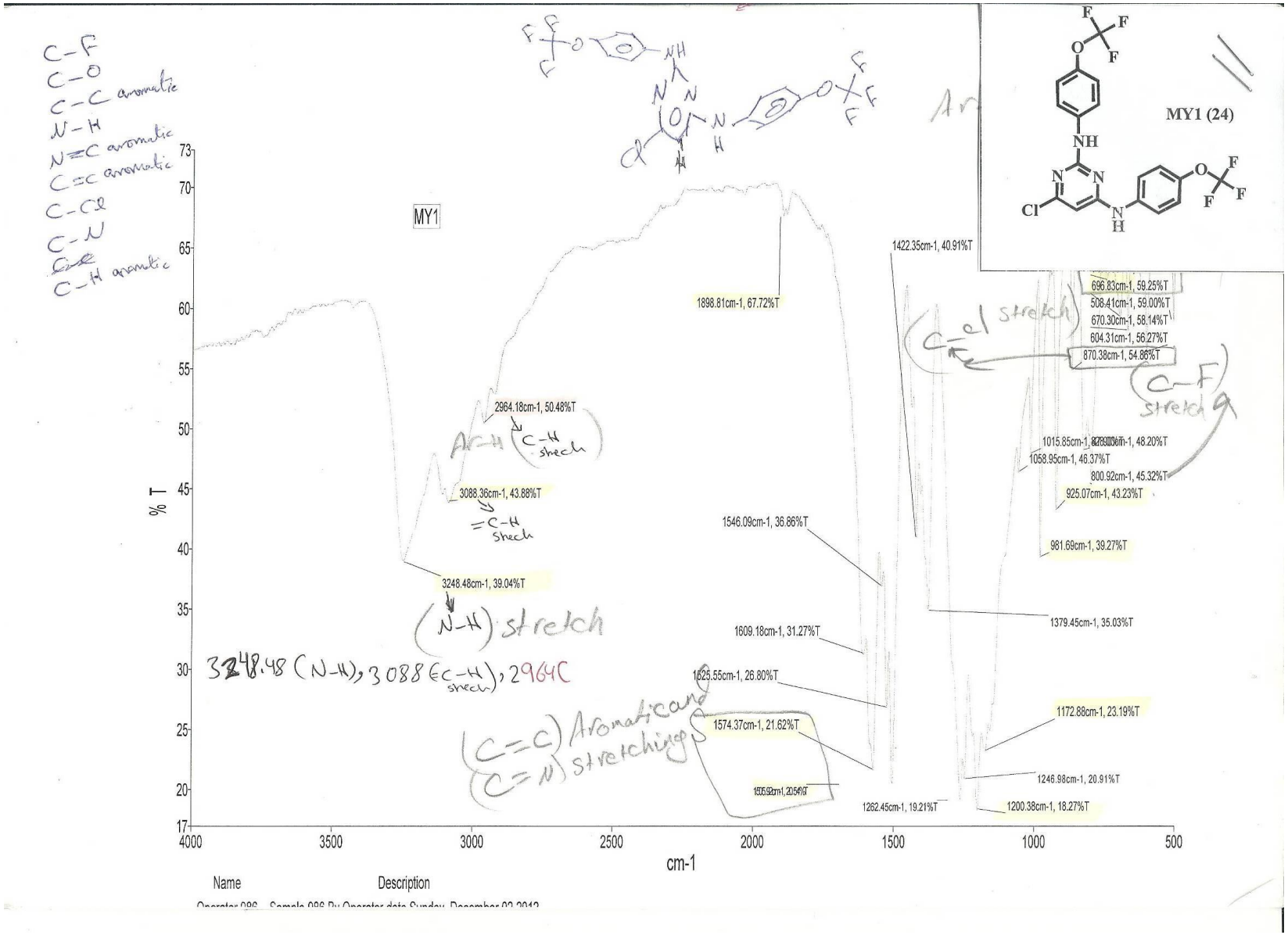
	RT	Area	% Area	Height
1	8.012	13670509	100.00	638401

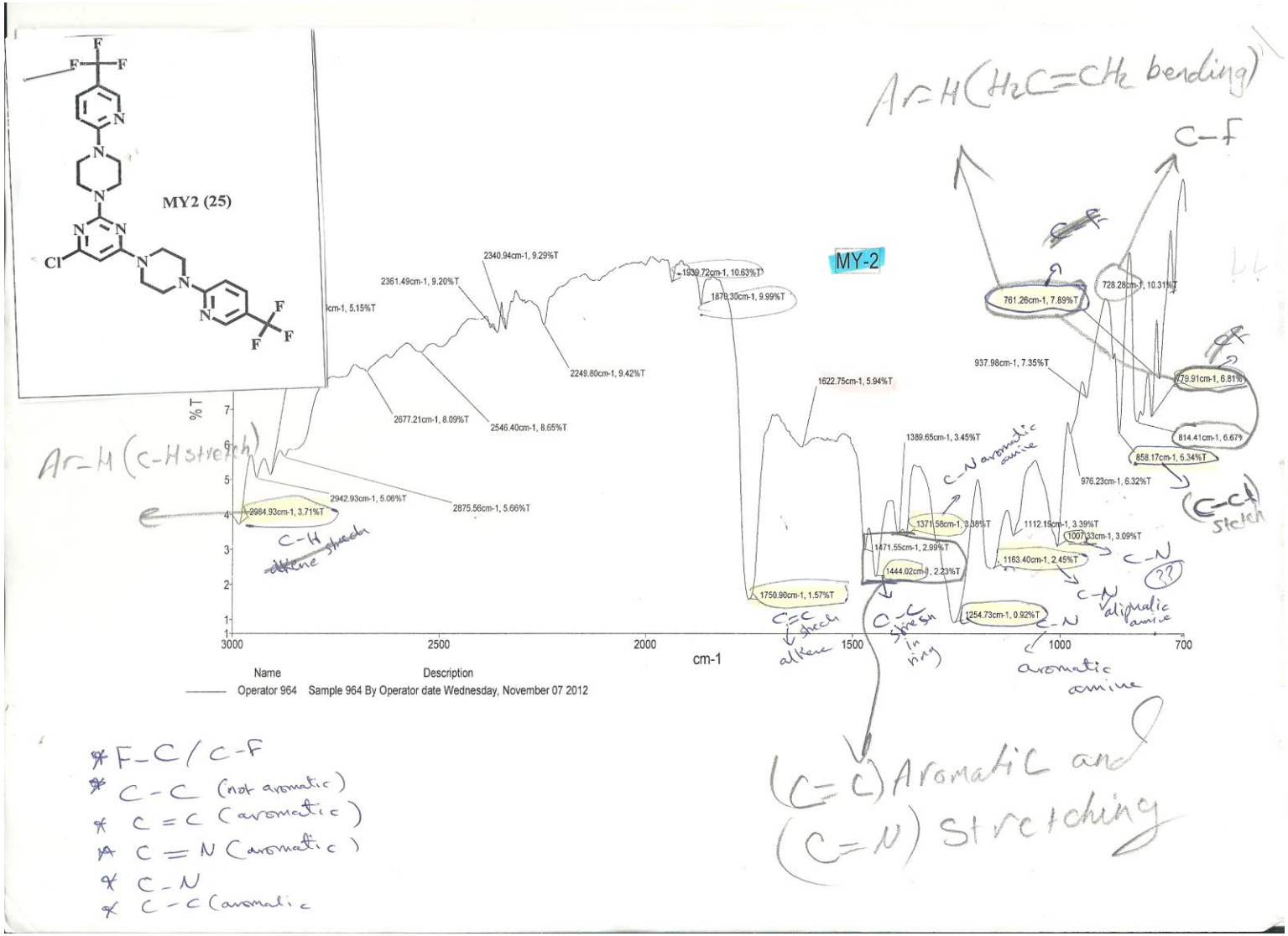
Reported by User: System
Report Method: Default Individual Report
Report Method ID: 1164
Page: 1 of 1

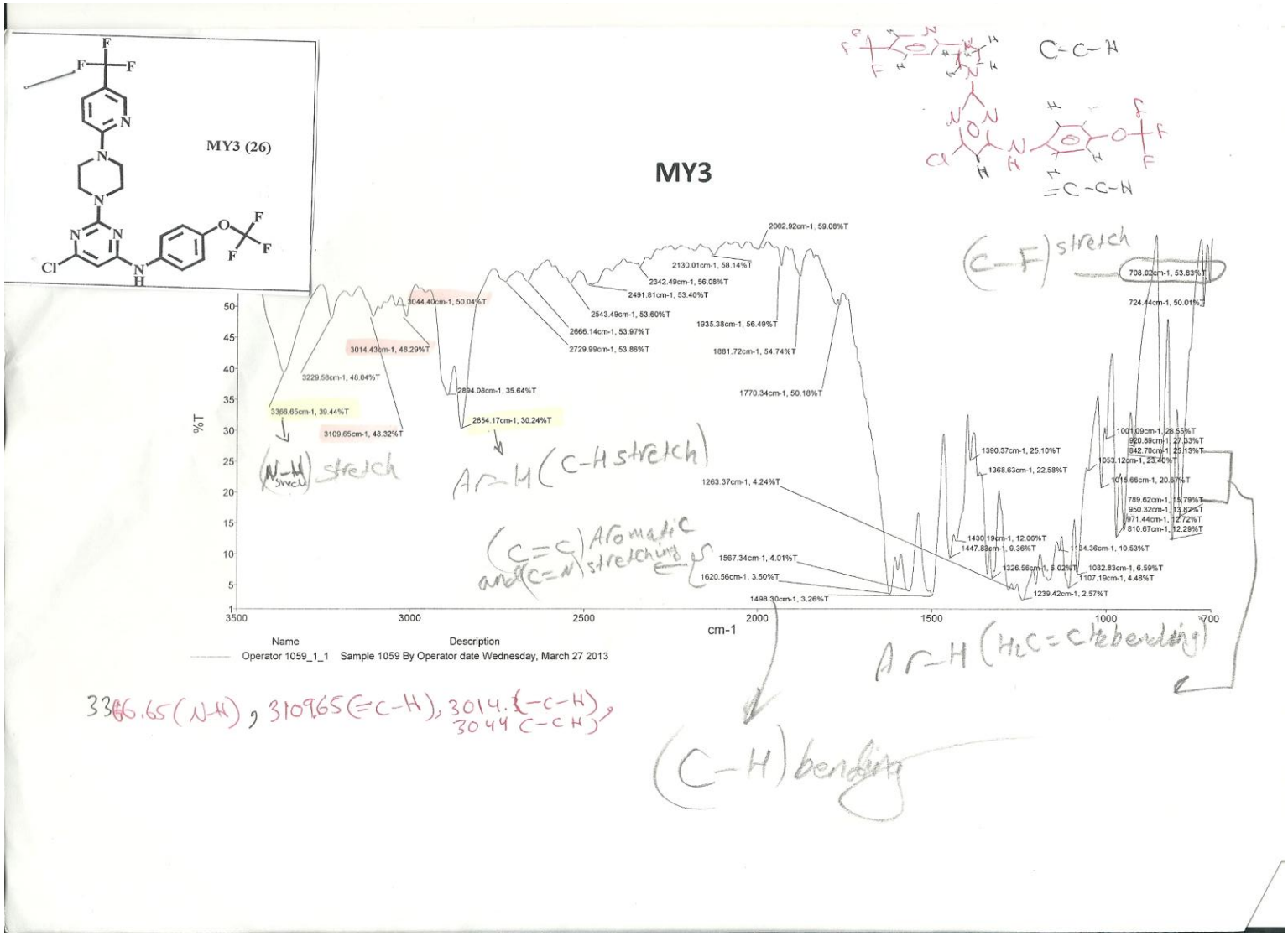
Project Name: yousef_najajreh
Date Printed: 2/16/2013
12:42:36 PM Israel

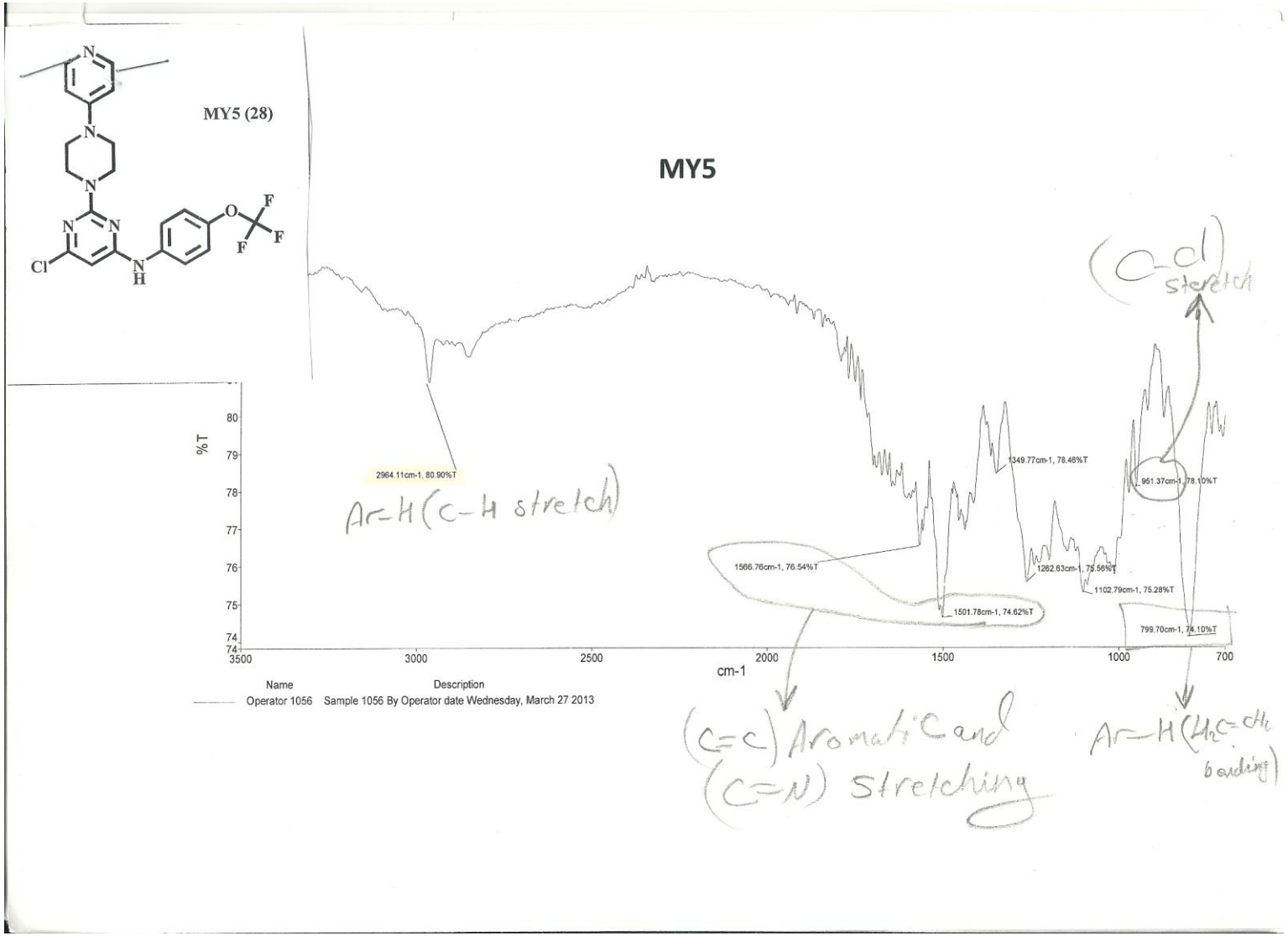


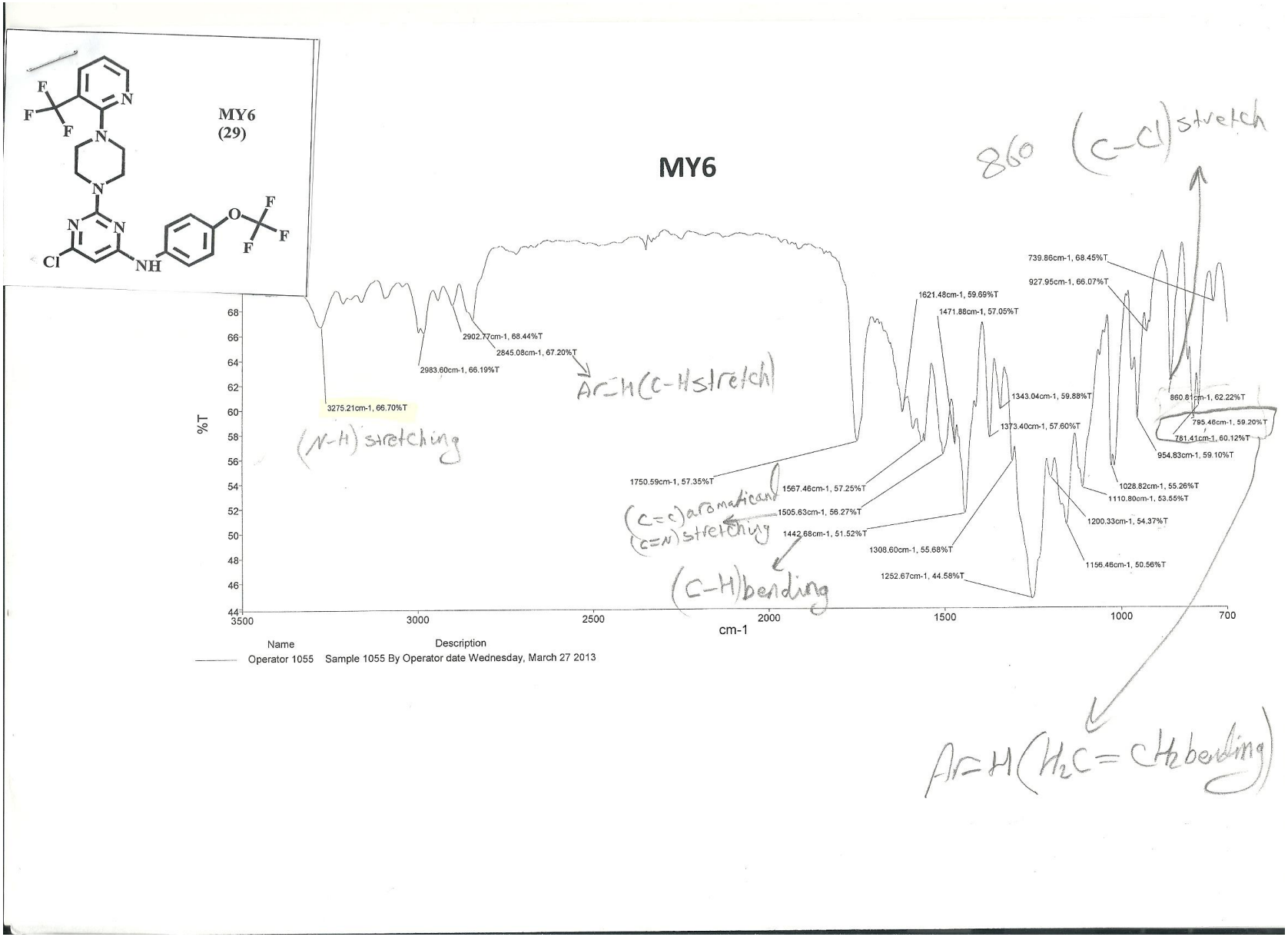

M3
C-Cl
C=N (aromatic)
C-C (aromatic)
N-H Stretching (3311)
C-N
C=C (aromatic)
C-O
C-F
C-H aromatic (3178.6)
C-H aromatic (3231.5 3178.6)
3231.5
3178.6
C-H stretch
3311.30cm-1, 83.42%T
N-H
(N-H) stretching
1735.46cm-1, 84.46%T
C=N aromatic
1624.32cm-1, 80.31%T
Benzene ring
1568.27cm-1, 77.54%T
1508.32cm-1, 77.78%T
(C=C) Aromatic and (C=N) stretching
C-C Stretch in aromatic ring
1409.73cm-1, 82.06%T
1370.82cm-1, 82.02%T
1453.20cm-1, 81.05%T
1252.39cm-1, 75.54%T
1118.32cm-1, 77.21%T
999.82cm-1, 79.23%T
967.28cm-1, 82.44%T
704.67cm-1, 85.08%T
C-F stretch
(C-Cl stretch
819.89cm-1, 81.22%T
838.56cm-1, 80.52%T
Ar-H
(H2C=CH2 bending)
819, 838 flat
(H2C=CH2
3311.3(N-H), 3178.6 (C-H stretch), 3231.5 (=C-H)
1735.46 (C=N aromatic),
1624 (C=C Benzene ring), 1508 (C-C) Stretching in aromatic ring,
% T
cm-1
Name
Description

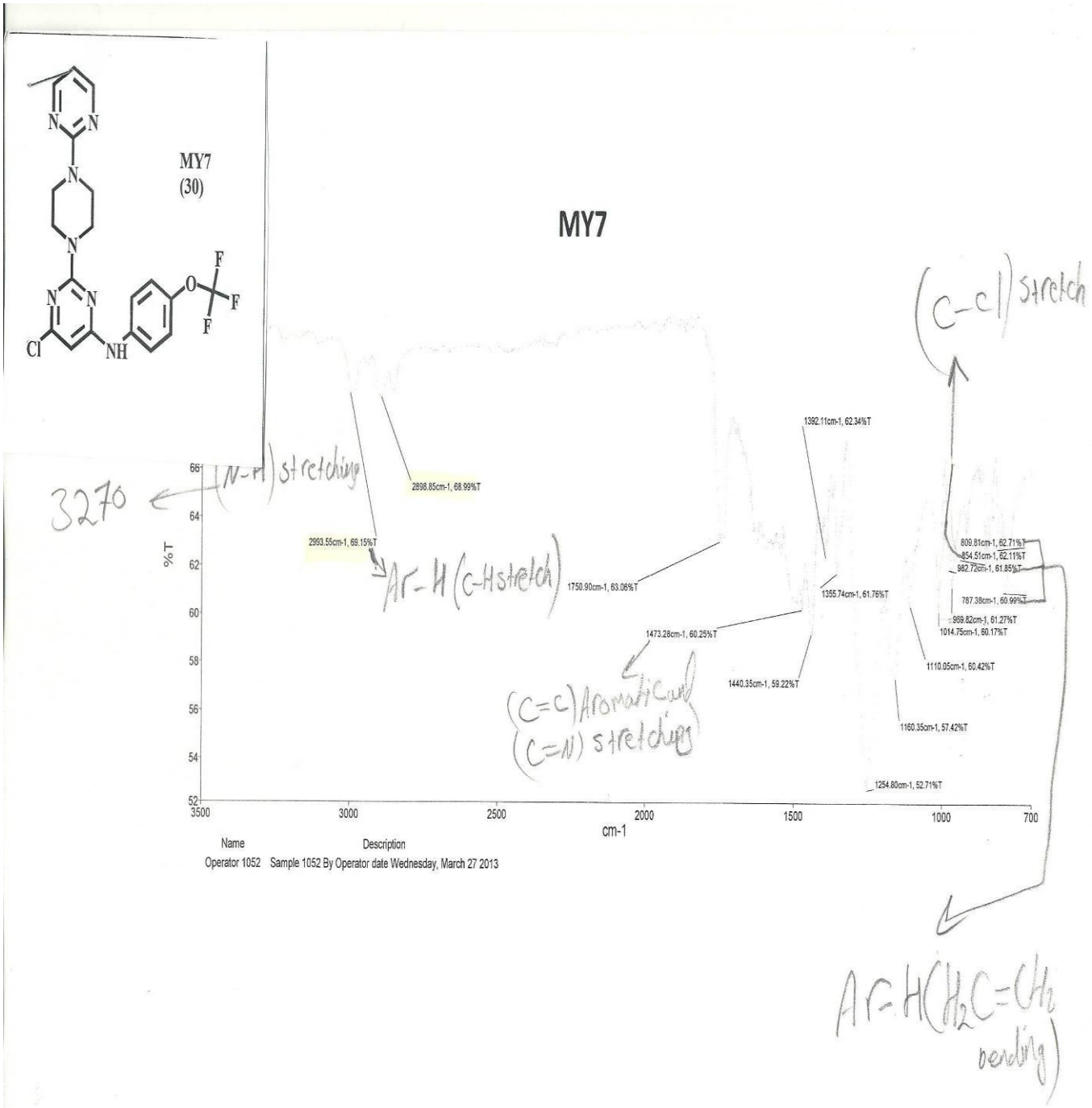

C-F
C-O
C-C aromatic
N-H
N=C aromatic
C=C aromatic
C-Cl
C-N
C-H aromatic
MY1
MY1 (24)
% T
cm-1
1898.81cm-1, 67.72%T
2964.18cm-1, 50.48%T
3088.36cm-1, 43.88%T
3248.48cm-1, 39.04%T
1546.09cm-1, 36.86%T
1609.18cm-1, 31.27%T
1625.55cm-1, 26.80%T
1574.37cm-1, 21.62%T
1422.35cm-1, 40.91%T
696.83cm-1, 59.25%T
508.41cm-1, 59.00%T
670.30cm-1, 58.14%T
604.31cm-1, 56.27%T
870.38cm-1, 54.86%T
1015.85cm-1, 48.20%T
1058.95cm-1, 46.37%T
800.92cm-1, 45.32%T
925.07cm-1, 43.23%T
981.69cm-1, 39.27%T
1379.45cm-1, 35.03%T
1172.88cm-1, 23.19%T
1246.98cm-1, 20.91%T
1262.45cm-1, 19.21%T
1200.38cm-1, 18.27%T
(N-H) stretch
3248.48 (N-H), 3088 (C-H stretch), 2964
(C=C) Aromatic and (C=N) stretchings
(C-Cl stretch)
(C-F stretching)
Name
Description


MY2 (25)
MY-2
Ar-H (H2C=CH2 bending)
C-F
Ar-H (C-H stretch)
C-H alkene stretch
C=C stretch alkene
C-C stretch in ring
C-N aromatic amine
C-N aliphatic amine
(C-Cl) stretch
(C=C) Aromatic and (C=N) Stretching
2984.93cm-1, 3.71%T
2942.93cm-1, 5.06%T
2875.56cm-1, 5.66%T
2677.21cm-1, 8.09%T
2546.40cm-1, 8.65%T
2361.49cm-1, 9.20%T
2340.94cm-1, 9.29%T
2249.80cm-1, 9.42%T
1939.72cm-1, 10.63%T
1870.30cm-1, 9.99%T
1622.75cm-1, 5.94%T
1750.90cm-1, 1.57%T
1471.55cm-1, 2.99%T
1444.02cm-1, 2.23%T
1389.65cm-1, 3.45%T
1371.58cm-1, 3.38%T
1254.73cm-1, 0.92%T
1163.40cm-1, 2.45%T
1112.19cm-1, 3.39%T
1007.33cm-1, 3.09%T
976.23cm-1, 6.32%T
937.98cm-1, 7.35%T
858.17cm-1, 6.34%T
814.41cm-1, 6.67%
779.91cm-1, 6.81%T
761.26cm-1, 7.89%T
728.28cm-1, 10.31%T
% T
cm-1
Name
Description
Operator 964 Sample 964 By Operator date Wednesday, November 07 2012
* F-C / C-F
* C-C (not aromatic)
* C=C (aromatic)
* C=N (aromatic)
* C-N
* C-C (aromatic

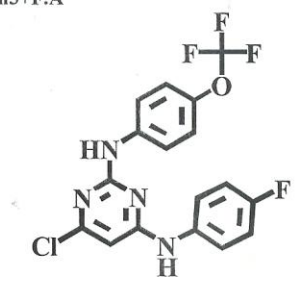

MY3 (26)
MY3
%T
cm-1
3366.65cm-1, 39.44%T
3229.58cm-1, 48.04%T
3109.65cm-1, 48.32%T
3044.40cm-1, 50.04%T
3014.43cm-1, 48.29%T
2894.08cm-1, 35.64%T
2854.17cm-1, 30.24%T
2729.99cm-1, 53.86%T
2666.14cm-1, 53.97%T
2543.49cm-1, 53.60%T
2491.81cm-1, 53.40%T
2342.49cm-1, 56.08%T
2130.01cm-1, 58.14%T
2002.92cm-1, 59.08%T
1935.38cm-1, 56.49%T
1881.72cm-1, 54.74%T
1770.34cm-1, 50.18%T
1620.56cm-1, 3.50%T
1567.34cm-1, 4.01%T
1498.30cm-1, 3.26%T
1447.88cm-1, 9.36%T
1430.19cm-1, 12.06%T
1390.37cm-1, 25.10%T
1368.63cm-1, 22.58%T
1263.37cm-1, 4.24%T
1239.42cm-1, 2.57%T
1184.36cm-1, 10.53%T
1107.19cm-1, 4.48%T
1082.83cm-1, 6.59%T
1326.56cm-1, 6.02%T
1053.12cm-1, 23.40%T
1015.66cm-1, 20.57%T
1001.09cm-1, 28.55%T
950.32cm-1, 13.82%T
971.44cm-1, 12.72%T
842.70cm-1, 25.13%T
810.67cm-1, 12.29%T
789.62cm-1, 15.79%T
724.44cm-1, 50.01%T
708.02cm-1, 53.83%T
Name
Description
Operator 1059_1_1 Sample 1059 By Operator date Wednesday, March 27 2013
(N-H) stretch
Ar-H (C-H stretch)
(C=C) Aromatic stretching and (C=N)
(C-F) stretch
Ar-H (H2C=CH bending)
(C-H) bending
3366.65 (N-H), 3109.65 (=C-H), 3014.43 (-C-H), 3044 (-C-H)
C-C-H
=C-C-H


MY5 (28)
MY5
%T
cm-1
2964.11cm-1, 80.90%T
1566.76cm-1, 76.54%T
1501.78cm-1, 74.62%T
1349.77cm-1, 78.46%T
1262.63cm-1, 75.56%T
1102.79cm-1, 75.28%T
951.37cm-1, 78.10%T
799.70cm-1, 74.10%T
Name
Description
Operator 1056 Sample 1056 By Operator date Wednesday, March 27 2013
Ar-H (C-H stretch)
(C-Cl) stretch
(C=C) Aromatic and (C=N) Stretching
Ar-H (H2C=CH bending)

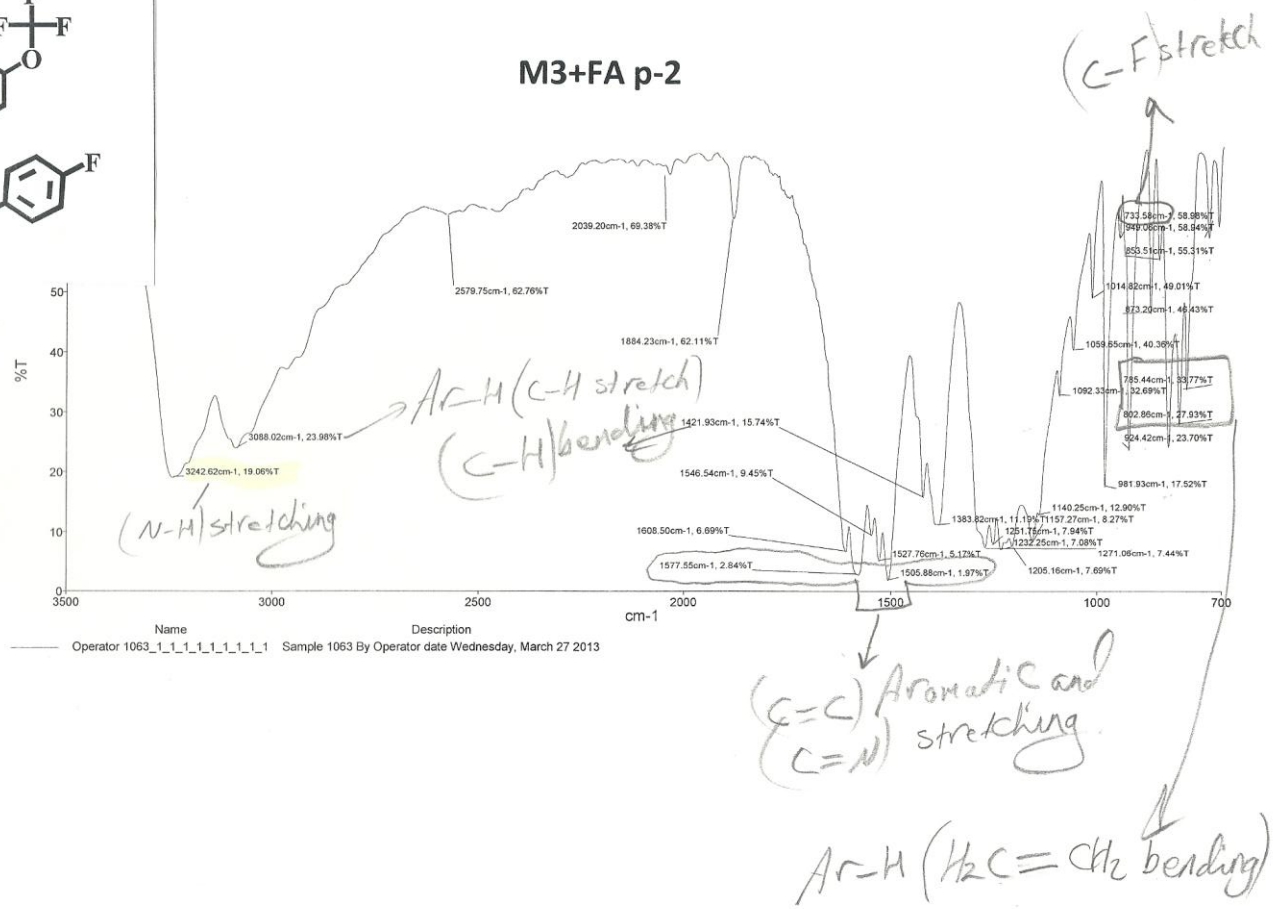

MY6
(29)
MY6
%T
cm-1
3275.21cm-1, 66.70%T
2983.60cm-1, 66.19%T
2902.77cm-1, 68.44%T
2845.08cm-1, 67.20%T
1750.59cm-1, 57.35%T
1621.48cm-1, 59.69%T
1567.46cm-1, 57.25%T
1505.63cm-1, 56.27%T
1471.88cm-1, 57.05%T
1442.68cm-1, 51.52%T
1343.04cm-1, 59.88%T
1373.40cm-1, 57.60%T
1308.60cm-1, 55.68%T
1252.67cm-1, 44.58%T
1200.33cm-1, 54.37%T
1156.46cm-1, 50.56%T
1110.80cm-1, 53.55%T
1028.82cm-1, 55.26%T
954.83cm-1, 59.10%T
927.95cm-1, 66.07%T
860.81cm-1, 62.22%T
795.46cm-1, 59.20%T
781.41cm-1, 60.12%T
739.86cm-1, 68.45%T
(N-H) stretching
Ar-H (C-H stretch)
(C=C) aromatic and (C=N) stretching
(C-H) bending
860 (C-Cl) stretch
Ar-H (H2C=CH2 bending)
Name
Description
Operator 1055 Sample 1055 By Operator date Wednesday, March 27 2013

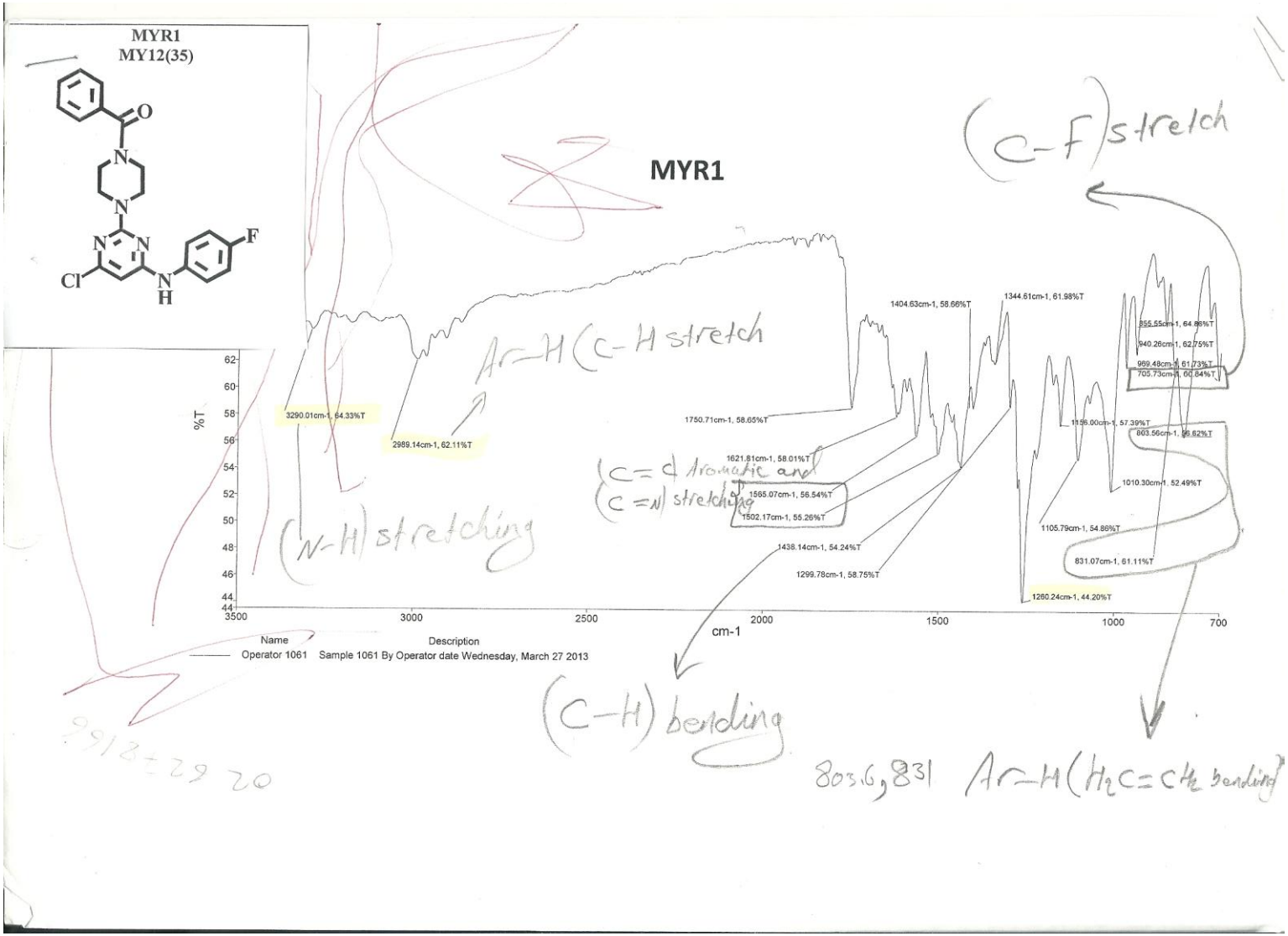

MY7
(30)
MY7
3270
(N-H) stretching
2898.85cm-1, 68.99%T
2993.55cm-1, 69.15%T
Ar-H (C-H stretch)
1750.90cm-1, 63.06%T
1392.11cm-1, 62.34%T
1355.74cm-1, 61.76%T
1473.28cm-1, 60.25%T
(C=C) Aromatic and
(C=N) stretching
1440.35cm-1, 59.22%T
1254.80cm-1, 52.71%T
1160.35cm-1, 57.42%T
1110.05cm-1, 60.42%T
1014.75cm-1, 60.17%T
999.82cm-1, 61.27%T
809.81cm-1, 62.71%T
854.51cm-1, 62.11%T
982.72cm-1, 61.85%T
787.38cm-1, 60.99%T
(C-Cl) stretch
Ar-H (H2C=CH2 bending)
%T
cm-1
Name
Description
Operator 1052 Sample 1052 By Operator date Wednesday, March 27 2013

MY10(33)
m3+F.A



M3+FA p-2




MYR1
MY12(35)
MYR1
(C-F) stretch
Ar-H (C-H stretch
(N-H) stretching
(C=C Aromatic and
(C=N) stretching
(C-H) bending
803.6, 831 Ar-H (H2C=CH2 bending
%T
cm-1
3290.01cm-1, 64.33%T
2989.14cm-1, 62.11%T
1750.71cm-1, 58.65%T
1621.81cm-1, 58.01%T
1565.07cm-1, 56.54%T
1502.17cm-1, 55.26%T
1438.14cm-1, 54.24%T
1404.63cm-1, 58.66%T
1344.61cm-1, 61.98%T
1299.78cm-1, 58.75%T
1260.24cm-1, 44.20%T
1196.00cm-1, 57.39%T
1105.79cm-1, 54.86%T
1010.30cm-1, 52.49%T
855.55cm-1, 64.86%T
940.26cm-1, 62.75%T
969.48cm-1, 61.73%T
831.07cm-1, 61.11%T
803.56cm-1, 59.62%T
705.73cm-1, 60.84%T
Name
Description
Operator 1061 Sample 1061 By Operator date Wednesday, March 27 2013

تصميم و تحضير مشتقات البيريميدين ثلاثية التفرع كمثبطات لبروتين مرافق المنشط لمستقبلات الاستروجين

اعداد : ميران "محمد جمال " طلال مسودى

اشراف : د. يوسف نجاجرة

ملخص:

يعتبر سرطان الثدي (BC) أحد أكثر أنواع السرطانات شيوعا وتشخيصا بين النساء. وتساهم إشارات ومستقبلات هرمون الاستروجين (ERα، ERβ) في تطور سرطان الثدي. حيث أن معظم هذه السرطانات تنشأ عن الزيادة المفرطة في مستويات الاستروجين الناتجة عن النشاط الزائد للتعبير الجيني لهرمون الاستروجين. ويعتمد العلاج في هذه مثل هذه الحالات على تقليل مستويات الاستروجين عن طريق استخدام مضادات هرمون الاستروجين (AES) و مثبطات إنزيم الأروماتيز (AIS) لإيقاف نشاط مستقبلات الاستروجين (ER) وكبح الخلايا السرطانية التي تعتمد في نموها وانتشارها على هرمون الاستروجين. على الرغم من أن هذه العلاجات لا تزال تمثل العلاج الأساسي لمرضى سرطان الثدي . الا أنها تفشل في كثير من الأحيان حيث تنشأ لدى المرضى مقاومة لهذه العلاجات تكون في غالبيتها غير قابلة للاستجابة .

في هذا البحث تم التركيز على تصنيع مثبطات بروتين مرافِق المنشِّط(coactivator) لمستقبلات الاستروجين. حيث تعمل هذه المركبات على وقف نشاط مستقبلات الاستروجين وكبح نمو خلايا سرطان الثدي بآلية مختلفة عن العلاجات السابقة. مما يقدم حلا لمشكلة المقاومة الحالية. مثبطات بروتين مرافق المنشط تعمل عن طريق اعاقة التغيرات التشكيلية اللازمة لارتباط الحمض النووي الريبوزي المنقوص الأكسجين (DNA) والتعبير الجيني(gene expression) لهرمون الاستروجين.

في هذا البحث تم تصميم وتصنيع مجموعة من المركبات بطريقة الاستبدال التسلسلي لذرات الكلور الموجودة في المركب (2,4,6-trichloropyrimidine) بمجموعات أخرى كالأمينات وغيرها. كما تمت تنقية هذه المركبات باستخدام تقنيات الفصل الكروماتوغ ا رفي، والتعرف عليها بواسطة .spectroscopy (^{1}H-NMR, ^{13}C-NMR, FT-IR and MS (ESI)) كما تم اختبارها للنشاط البيولوجي ضد خلايا سرطان الدم النخاعي الحاد (Molm-13). حيث استخدم نوعين من هذه الخلايا لتقييم دور البروتين p53. مرة باستخدام الخلايا مع ناقلات فارغة والآخرى مع الخلايا مع (Sh-البروتين p53). لقد تمت تحديد سلامة الخلايا باستخدام تقنية 1-WST.

بينت النتائج الأولية لاختبار فعالية هذه المركبات على الخلايا السرطانية (Molm-13) أن المركبات **(MY12 (35)** و**((MY3 (26)** أظهرت نشاطا فعال في تثبيط انتشار وتكاثر خلايا النوعين المستخدمين من خلايا (Molm-13) في حين أن المركب **((MY2(25)** لم يظهر فعالية ملحوظة في تثبيط أي نوع من خلايا (Molm-13).