Design, Synthesis and Biological Evaluation of Nitrogenous Analogues as Anticancer Agents

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CERTIFICATE

This is to certify that the thesis entitled "**Design, Synthesis and Biological Evaluation of Nitrogenous Analogues as Anticancer Agents**" and submitted by **SURESH N** ID No: **2011PHXF034H** for award of Ph.D. of the Institute embodies original work done by him under my supervision.

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Abstract

In the present study, we have focused on achieving promising anticancer compounds by design, synthesis and antiproliferative evaluation of synthesized compounds based on reported promising anticancer agents. The synthesized compounds were subjected to study anticancer activity against various cancer cell lines.

In chapter 3, a series of sixty four fluoroquinolone analogues have been synthesized, and cytotoxic evaluations of these molecules on human cancer cell lines by MTT assay, cell proliferation assay were done. Among the synthesized compounds **3j**, **3t**, **6o**, **8r** and **8t** exhibited good anticancer activity.

In chapter 4 a series of twenty eight novel 2-(4-aminophenyl)benzothiazole analogues have been synthesized, characterized and evaluated their antiproliferative activity against A549, HeLa and MDA MB-231 using sulforhodamine-B assay method. Among the synthesized compounds **13g**, **13j**, **15k** exhibited maximum growth inhibitory activity.

In chapter 5, series of thirty two novel pyrrolo[2,3-*b*]pyridine analogues have been synthesized, characterized and evaluated their antiproliferative activity against A549, HeLa and MDA MB-231, using sulforhodamine B assay method. Among the synthesized compounds **20c**, **20d**, **20e**, **20h**, **20k**, **20m**, **20n**, **20q**, **20r**, **20f**, **20j**, **20g** and **20k** exhibited maximum growth inhibitory action at lower micro molar concentration.

In chapter 6, series of thirty seven novel 1,3,5-triazine analogues have been synthesized, characterized and evaluated their antiproliferative activity against HeLa, HepG2, A549, and MCF-7, using sulforhodamine B assay method. Among the synthesized compounds **30j** exhibited comparable inhibitory action.

In chapter 7, series of eight novel novel 6-(4-((substituted-1*H*-1,2,3-triazol-4-yl)methyl) piperazin-1-yl)phenanthridine analogues and their evaluation as anticancer agents against four cancer cell lines by MTT assay. Among the synthesized compounds **38g** & **38h** showed good activity against all the test cell lines.

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List of Abbreviations

µg	:	Microgram
µM	:	Micromolar
^{13}C NMR	:	Carbon Nuclear Magnetic Resonance
^{1}H NMR	:	Proton Nuclear Magnetic Resonance
br	:	Broad singlet
$CDCl_3$	:	Chloroform- deuterated
CP		Ciprofloxacin
CtDNA	:	Calf thymus deoxyribonucleic acid
CuAAC		Copper-catalyzed azide-alkyne cycloaddition
$CuSO_4.5H_2O$	:	Copper sulphate pentahydrate
d	:	Doublet
DCM	:	Dichloromethane
dd	:	Doublet of doublet
DIPEA	:	*N*,*N*-Diisopropylethylamine
DMF	:	*N*,*N*-Dimethylformamide
DMSO	:	*N*,*N*-dimethylsulfoxide
DMSO-d_6	:	Dimethyl sulphoxide deuterated
DNA	:	Deoxyribonucleic acid
Dox	:	Doxorubicin
EB	:	Ethidium bromide
EDC.HCl	:	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI	:	Electron Spin Ionisation
Et_3N	:	Triethylamine
EtOAc	:	Ethyl acetate
FBS	:	Foetal bovine serum
FDA	:	Food and Drug Administration
FQ	:	Fluoroquinolone
g	:	Gram

GI_{50}	:	Growth inhibition
h	:	Hour
H_2O	:	Water
HEK	:	Embryonic kidney cell line
HOBt	:	Hydroxybenzotriazole
HRMS	:	High-resolution mass spectra
Hz	:	Hertz
IC_{50}	:	Minimum Inhibitory Concentration
IR	:	Infrared Spectroscopy
J	:	Coupling constant
KBr	:	Potassium Bromide
KI	:	Potassium Iodide
m	:	Multiplet
m.p.	:	Melting point
MeOH	:	Methanol
mg	:	Milligram
MHz	:	Megahertz
mmol	:	Milli molar
MS	:	Mass spectrometry
MTT assay	:	[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay
MW	:	Microwave
Na_2CO_3	:	Sodium carbonate
nm	:	Nano molar
PC-3	:	Prostate cancer cell line
PDB	:	Protein Data Bank
PPA	:	Polyphosparicacid
RNA	:	Ribo Nucleic Acid
s	:	Singlet
SP	:	Standard Precision
SRB assay	:	Sulforhodamine-B assay
t	:	Triplet

^{t}BuOH	:	Tertiary butanol
TEA	:	Triethylamine
TLC	:	Thin-layer chromatography
tt	:	Triplet of triplet
UA	:	Ursolic acid
UV	:	Ultra Violet

Chapter I

Introduction

Introduction Chapter 1

1. General introduction

Cancer is a cluster of diseases characterized by uncontrolled augmentation and spread of abnormal cells. The human body is made up of trillions of living cells, these normal cells grow, divide to make new cells, and die in an orderly way. Cancer starts while cells in a part of the human body begin to grow out of control. Cancer cell augmentation is dissimilar from common cell growth. Cancer cells are continued to grow and form new, abnormal cells. Cancer cells grow out of control, invade to other tissues and make a normal cell as a cancerous cell [1]. Cancer can occur due to external and internal factors. External factors include tobacco, infectious organisms, an unhealthy diet and environmental exposures to different types of chemicals and radiation. Internal factors are, like inherited genetic mutations, hormones, and immune conditions. These factors may act together or in sequence to cause cancer.

Cancer is a foremost public health problem in the United States and many other parts of the world. It is presently the second leading reason of death in the United States, and is expected to surpass heart diseases as the leading cause of death in the next few years. In 2015, almost 171,000 of the estimated 589,430 cancer deaths in the US will be caused by tobacco smoking. Cancer most commonly develops in older people; 78% of all cancer diagnoses are 55 of years age or older. According to the statistics obtained by the U.S National Cancer Institute, 1 in 2 men in the United States have a lifetime risk of developing cancer while this risk is 1 in 3 for women [2, 3]. These statistics emphasize the need for continued development and progress in the field of cancer research.

In India, the total cancer cases are expected to go up from 979,786 cases in the year 2010 to 1,148,757 cases in the year 2020. The tobacco-related cancers for males are expected to go up from 190,244 in the year 2010 to 225,241 in the year 2020. Similarly, the female cases will go up from 75,289 in year 2010 to 93,563 in the year 2020. Gynecological related cancers are estimated to go up from 153,850 in 2010 to 182,602 in 2020 [4].

1.1. Metastasis:

A cancer that has spread from the one place where it first started in human body to another place in the body is called metastatic cancer. The process by which cancer cells spread to other parts of the body is called metastasis [5].

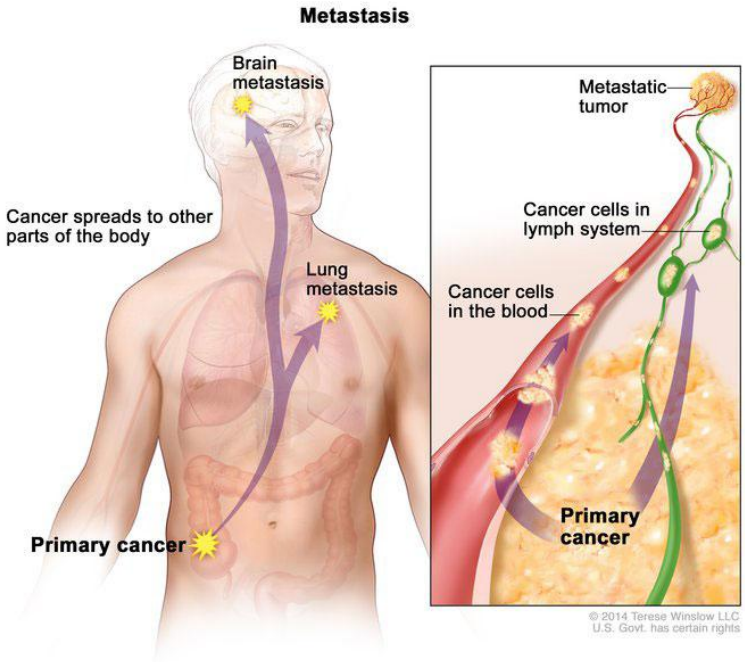

Figure 1.1: Metastasis process

1.2. Carcinogenesis

Carcinogenesis can be defined as the creation or formation of cancer. In most cases, cellular transformation is a result of activation of oncogenes or suppression of tumor suppressor genes. Cellular oncogenes, also called proto-oncogenes, are normal genes required for important functions in the cell. These genes however, can be transformed into oncogenes by retro-viruses resulting in abnormal cellular proliferation [6, 7]. On the other hand, tumor suppressor genes or anti-oncogenes limit cellular transformation. These genes encode proteins that inhibit cell cycle progression, promote DNA damage repair and bring about cell death in the event of mutations or stress [8, 9].

Carcinogenesis is a multistage process that develops through three phases: Initiation, promotion and progression [10]. Initiation involves an irreversible change in the cell which is generally an

insult to the DNA of the cell. Chemicals such as aromatic hydrocarbons, radiation (ionizing and ultraviolet) or biological agents such as retroviruses act as carcinogens to initiate cancer.

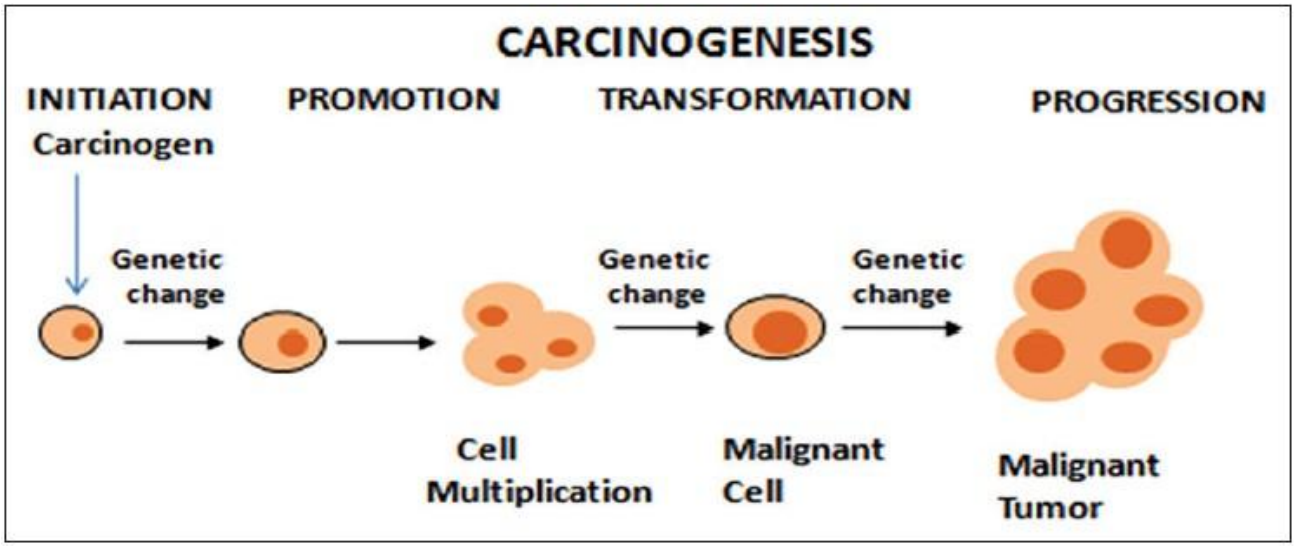

Figure 1.2: Carcinogenesis process

These carcinogens can cause multiple mutations in the DNA of the cells such that the DNA repair machinery is impaired. As a result, cell cycle checkpoints are deregulated and the cell divides and proliferates despite the mutations. Tumor promotion involves the proliferation and expansion of the mutant and genetically unstable cell and accumulation of further mutations with each round of cell division such that the resulting population of cells is capable of surviving in normally unsuitable cellular environments [11]. The progression step comprises of tumor cells that have attained malignant properties, invasiveness and metastatic capabilities. Of note is the fact that mutations that occur during the process of carcinogenesis do not just involve the genetic alteration (deletion, translocation, point mutation, duplication or amplification) of oncogenes or tumor suppressor genes, but can also be epigenetic changes such as modifications of gene promoters by acetylation/ deacetylation or methylation/demethylation [12, 13]. These causal factors may act together or in sequence to initiate or promote the development of cancer.

Century leading to the advanced understanding of anatomy and physiology did the origin and progression of cancer become clear. We now find ourselves in the fortunate position of

understanding a portion of the molecular mechanisms that regulate cancer initiation and progression. This knowledge has led to the development of modern cancer therapies that effectively treat many cancers. However, treatment options remain limited and deaths attributed to cancer are still a major cause of mortality throughout the world. To fully combat cancer, continued research into the molecular causes and novel therapies must be completed. A task which seems to become more tangible with each passing day as new technologies for this research becomes available.

1.3. Literature review on anticancer agents:

The innovation and progress of small molecule cancer drugs has been revolutionized over the previous decade. Radiation therapy and surgery as way of cancer treatment are only successful at the early stages of the cancer disease. Chemotherapy, in contrast, is the mainstay in the treatment of malignancies because of its ability to cure widespread cancer. The effort of anticancer drug discovery seems to be promising since it is believed that small molecules have been optimized during evolution.

The chemotherapy for cancer started in the 1940s with nitrogen mustards, which are very powerful alkylating agents and antimetabolites. With these initial treatments, a large number of further anticancer drugs have been developed [14]. Chemotherapy involves the use of low-molecular-weight drugs to selectively destroy tumor cells or at least bind their proliferation. The cytotoxic agents act on tumor cells as well as normal human cells so side effects occur in human body like bone marrow suppression, gastrointestinal tract lesions, hair loss, nausea, and the development of clinical resistance [15]. Anticancer drugs can be classified according to their mechanism of action, such as antimetabolites, DNA-interactive agents, antitubulin agents, molecular targeting agents, hormones, monoclonal antibodies and other biological agents [15].

1.3.1. Antimetabolites:

Antimetabolites are the old anticancer drugs, which interact with essential biosynthesis pathways. Pyrimidines are incorporated into cell components to disrupt the synthesis of nucleic acids. The chemical structures of pyrimidine containing antimetabolites are shown in **Figure 1.3**

[16]. 5-Azacytidine is used for the treatment of myelodysplastic syndromes [17]. Cytarabine is one of the most effective single agents available for treating acute myeloblastic leukaemia, although myelosuppression is a major side effect [18].

5-Fluorouracil is a broadly used cytotoxic agent for the treatment of breast tumours, gastrointestinal tract cancer and advanced colorectal cancer. It is also effective for certain skin cancers by topical administration. The main side effects include myelosuppression and mucositis [15]. Tegafur is given orally for metastatic colorectal cancer [19].

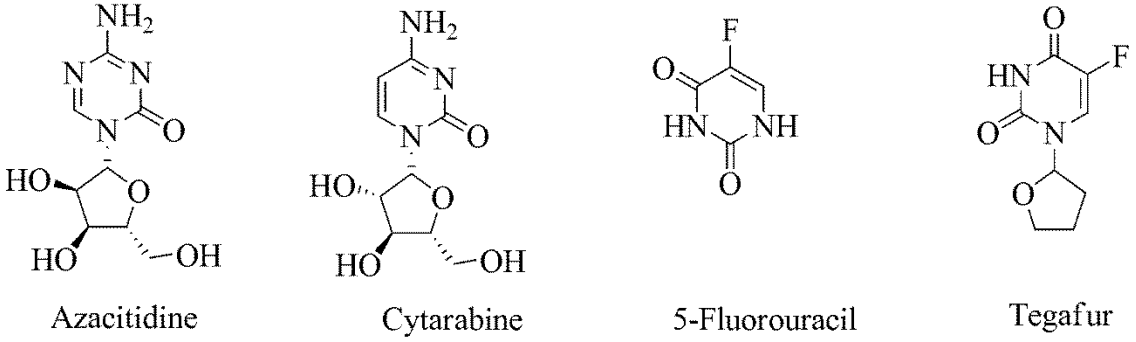

Figure 1.3: Pyrimidine containing anticancer agents

Purines disrupt the synthesis of nucleic acids by their incorporation into cell components. Mercaptopurine is typical purine analogue. Mercaptopurine is a purine sulfur derivative approved as an antitumor drug by Food and Drug Administration (FDA) in 1953 [20] and used for therapy of acute leukaemia. Currently children's leukemia is treated with mercaptopurine associated to other anti-tumor drugs [21].

Thioguanine is used orally to induce remission in acute myeloid leukaemia and it is an inhibitor in concentration-dependent fashion [22]. Azathioprine, an immunosuppressant agent, is a useful antileukaemic drug and is metabolised to 6-mercaptopurine [15, 23]. Fludarabine is also used for chronic lymphocytic leukaemia after failure of an initial treatment with an alkylating agent [15]. The chemical structures of purine containing antimetabolites are shown in **Figure 1.4** [16].

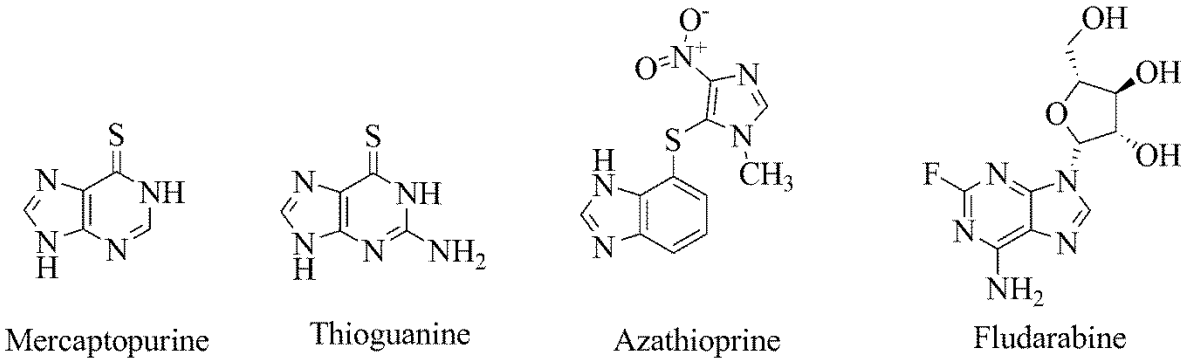

Figure 1.4: Purine containing anticancer agents

1.3.2. DNA interactive agents:

DNA interactive agents are most significant anticancer drug families, acting through a diversity of mechanisms.

1.3.2.1. Cross linking agents:

Platinum complexes are the most generally used class of drugs in cancer treatment and possess a prominent activity in various cancer types. Platinum anticancer agents are depicted in **Figure 1.5**. Cisplatin is one of the most successful and widely used chemotherapeutics for the patients affected by various human malignant solid, metastatic tumors, esophageal cancer [24], testicular and ovarian cancers [15]. The related platinum analogues carboplatin and oxaliplatin were developed later to reduce the side effects of cisplatin. Carboplatin is used in the treatment of advanced ovarian cancer and lung cancer.

Carboplatin displays a more acceptable toxicological profile due to the higher stability of the chelating 1,1-cyclobutanedicarboxylato ligand when compared to the chloride ligands in cisplatin [25, 26]. Oxaliplatin is a third-generation platinum derivative for the treatment of metastatic colorectal cancer. Oxaliplatin, is approved by the US Food and Drug Administration in 2002, after success of the other platinum based drugs, cisplatin and carboplatin. Oxaliplatin has been widely regarded as an effective chemotherapeutic agent for the treatment of cisplatin resistant cancer [27, 28]

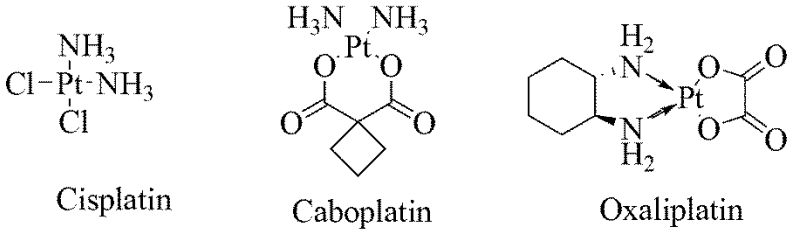

Figure 1.5: Platinum complexes as anticancer agents

Chlorambucil is an alkylating agent of the nitrogen mustard group and is used as cytostatic drug in ovarian cancer therapy [16, 29]. The alkylating agents form adducts with DNA and also form adducts with RNA and protein which are likely to contribute to the overall cytotoxicity [30, 31]. Melphalan is an anticancer drug used for the treatment of multiple myeloma, solid tumours like breast and ovarian tumours [32]. Estramustine is a conjugate consisting of chlormethine chemically linked to an oestrogen moiety and is used for patients with metastatic prostate cancer as well as breast cancer and malignant glioma [33, 34]. Nitrogen mustard anticancer agents are depicted in **Figure 1.6**.

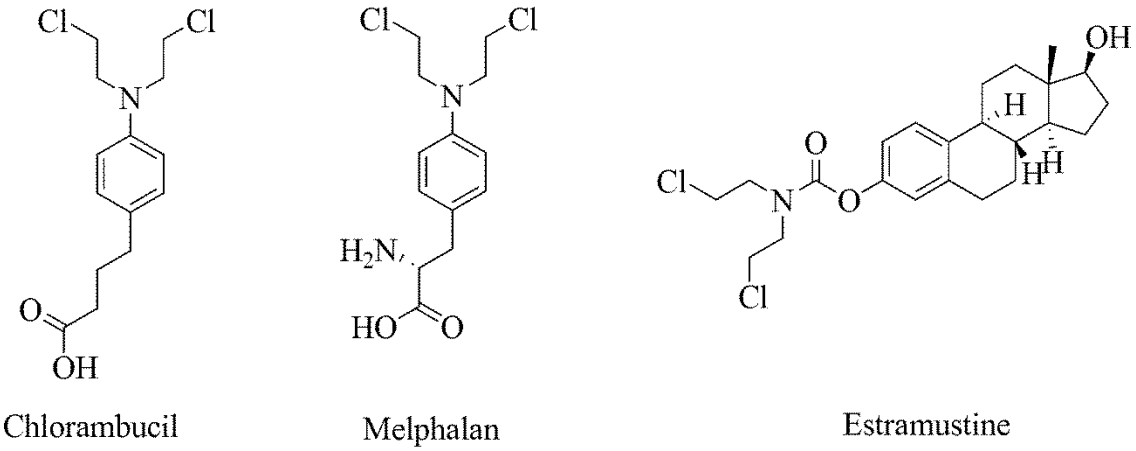

Figure 1.6: Nitrogen mustard anticancer agents

Among anticancer agents, nitrosoureas are tremendously active class of alkylating compounds that have extensive clinical use in the treatment of brain tumours, melanomas and various leukemias. Lomustine is a nitrosurea analog and oral alkylating drug commonly used for

brain tumor, and Hodgkin's disease. Lomustine is a standard control drug for clinical trials in recurrent glioblastoma [35]. Nitrosourea based anticancer agents are depicted in **Figure 1.7**. Carmustine has a comparable activity and toxicity profile to lomustine and might deactivate the molecular pathways related to p53 in GBM U87MG cells [36, 37]. Fotemustine is a third generation nitrosourea, used clinically against disseminated malignant melanoma. Its clinical application is somewhat limited by its toxicity and also by acquired resistance of melanoma cells to this antineoplastic agent [38].

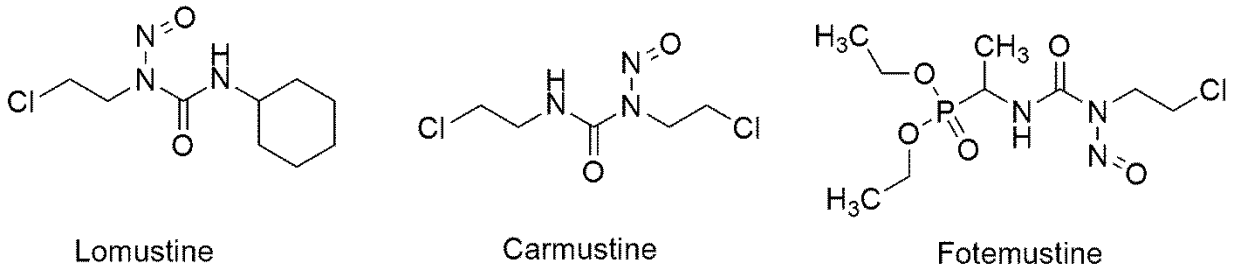

Figure 1.7: Nitrosoureas as anticancer agents

1.3.2.2. Intercalating agents:

Anthracyclines are a cluster of antitumor agents consisting of a planar anthraquinone nucleus attached to an amino-containing sugar. Doxorubicin (Dox), daunorubicin, and aclarubicin are natural products extracted from *Streptomyces peucetius* or *Streptomyces galilaeus*, while epirubicin and idarubicin are semisynthetic analogues.

Doxorubicin is an anthracycline antibiotic commonly used as an anticancer agent in the treatment of leukemia, breast carcinoma, and other solid tumors [39]. Although Dox is also used for treating other tumors like ovarian carcinoma, liver cancer, and stomach cancer, it is not the first choice in the clinic for these cancers due to the emergence of drug resistance [40]. Dox does not show high antiproliferative activity against ovarian carcinoma cell line SK-OV-3. It shows IC_{50} value of 5μM following 48 h of incubation [41].

Daunorubicin is an important agent in the treatment of acute lymphocytic and myelocytic leukaemia, while aclarubicin is used as a second-line treatment for acute nonlymphocytic

leukaemia [15, 16]. Epirubicin, a semisynthetic analogue of Dox differing only by its stereochemistry, is similar in terms of efficacy for the treatment of breast cancer and a less cardiotoxic analogue characterized by an axial-to-equatorial epimerization of the hydroxyl group at C-4 in the amino sugar bound to the tetracyclic ring [42].

Idarubicin is used in advanced breast cancer after failure of first-line chemotherapy and in acute nonlymphocytic leukaemia, more importantly, idarubicin has demonstrated a 10-fold higher cytotoxic activity than daunorubicin in cultured human cancer cells [43]. Idarubicin is more lipophilic than either daunorubicin or Dox [44, 45]. Anthracyclin anticancer agents are showed in **Figure 1.8**.

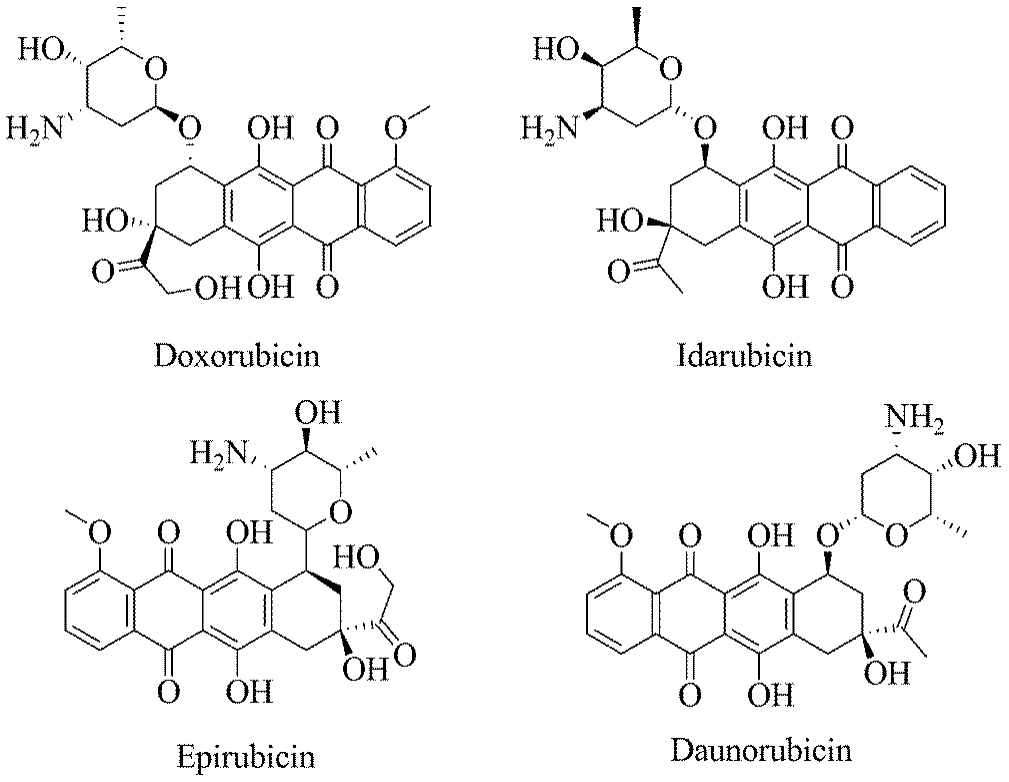

Figure 1.8: Anthracyclins as anticancer agents

1.3.2.3.Topoisomerase inhibitors:

1.3.2.3.1. Topoisomerase I inhibitors:

Camptothecin is a natural product, a cytotoxic quinoline based alkaloid with a five ring system extracted from the bark of the Chinese *Camptotheca* and the Asian *Nothapodytes* trees. The main disadvantage of camptothecin is low water solubility and severe side effects [16]. Topoisomerase I inhibitors are shown in **Figure 1.9**. Recently several derivatives of camptothecin were prepared with improved solubility. Topotecan is a semi-synthetic water soluble analogue of camptothecin, and has been recently approved for the treatment of relapsed small cell lung cancer in 2007 [46]. Irinotecan is licensed for metastatic colorectal cancer. Patient with a colorectal cancer who developed a type 1 hypersensitivity reaction to irinotecan was fruitfully treated with a rapid drug desensitization protocol [47].

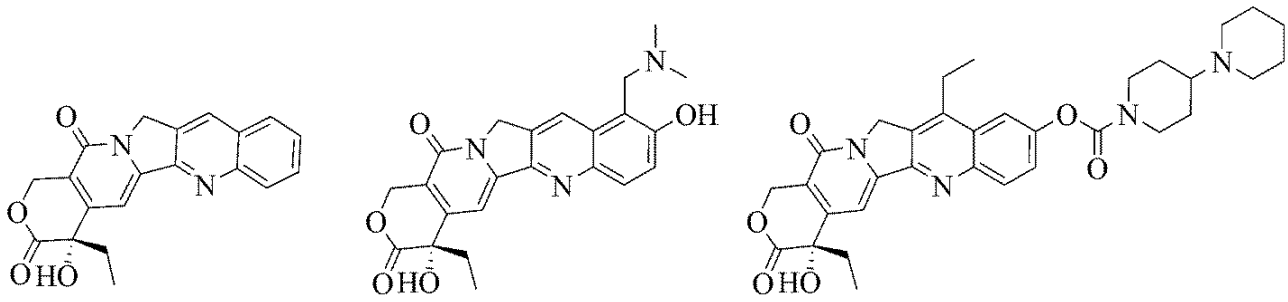

Figure 1.9: Topoisomerase I inhibitors

1.3.2.3.2. Topoisomerase II inhibitors:

Topoisomerase inhibitors inhibit the responsible enzymes for the cleavage, annealing, and topological state of DNA. Amsacrine is an acridine based structure, it is in multiple clinical trials for the treatment of hematological cancers and is used for treatment of refractory acute lymphocytic and nonlymphocytic leukemias as well as Hodgkin's and non-Hodgkin's lymphomas [48].

Etoposide is one of the most effective agents for treating small-cell bronchial carcinoma. It can also be used in treating wide range of solid and hematologic malignancies. The toxic effects of this drug include nausea and vomiting, myelosuppression, and alopecia [49, 50]. Topoisomerase II inhibitors are shown in **Figure 1.10**.

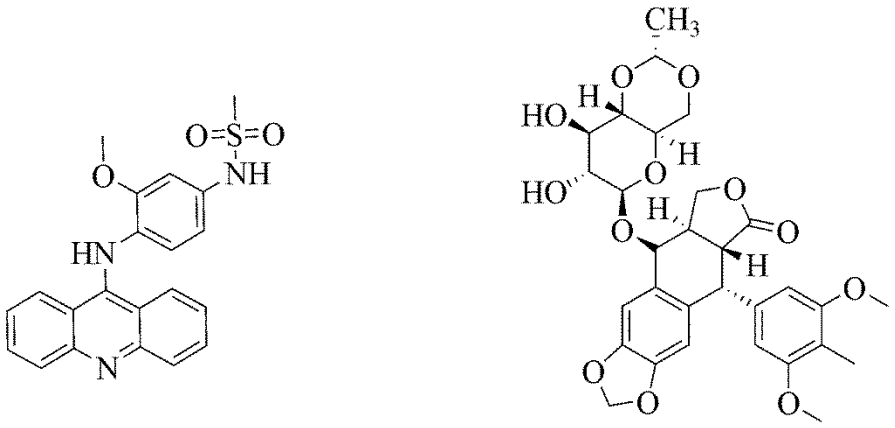

Figure 1.10: Topoisomerase II inhibitors

1.3.3. Antitubulin agents:

Antitubulin agents interfere with the microtubule dynamics (spindle formation or disassembly) and block the division of the nucleus and lead to cell death. Paclitaxel is a tetracyclic diterpene, extracted from needles and bark of *Taxus brevifolia*, the Pacific yew tree. Pure paclitaxel was isolated in 1966, its structure was published in 1971 and it did not appear in clinical practice until the 1990's. Paclitaxel, potentiates tumor destruction via apoptosis and is used as first line therapy for advanced non-small cell lung cancer [51].

Docetaxel is more recently introduced semi synthetic, second generation taxane analogue. Docetaxel is licensed for initial treatment of advanced breast cancer and can encourage cell apoptosis by varying the expression and phosphorylation of members of the Bcl-2 family of proteins [52]. Antitubulin agents are shown in **Figure 1.11**.

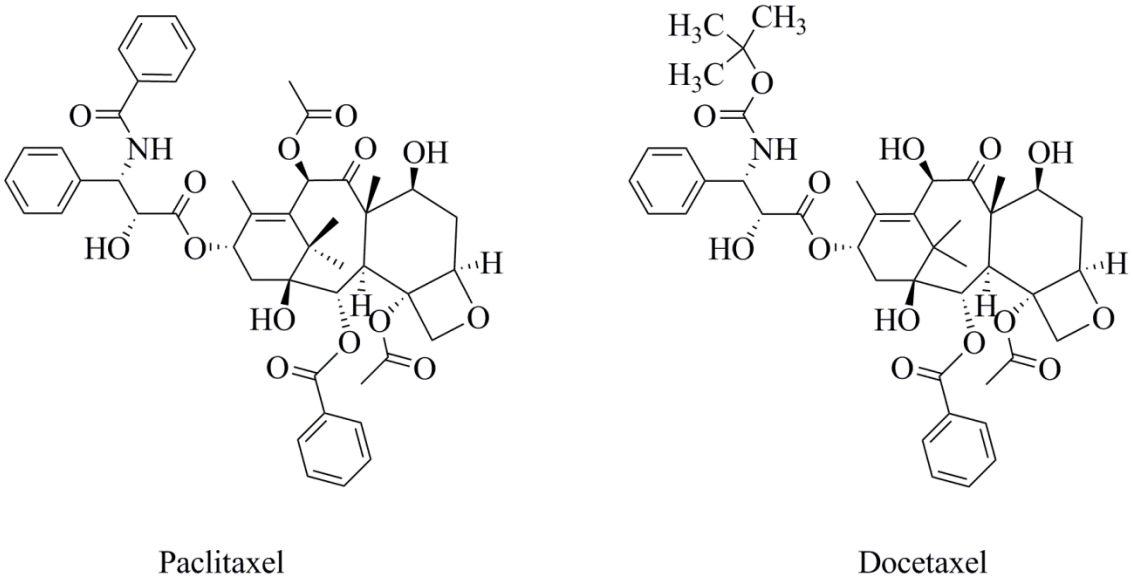

Figure 1.11: Structures of antitubulin agents

Based on these anticancer agents recently various anticancer analogs have been developed and evaluated for their anticancer activity. Our interest is the synthesis of various heterocyclic compounds and evaluation of their antiproliferative effect. The molecules designed with hybridization or derivatization approaches were taken up for the synthesis of novel molecules. Wherever possible we carried out reactions using less exposure of hazardous chemicals/vapours to the environment. Most of the synthesized molecules were purified by flash chromatography with lesser amount of solvents to maintain eco-friendly conditions.

From the literature survey of recently synthesized anticancer agents, we preferred mainly five heterocyclic scaffolds, viz., fluoroquinolone (**A**), 2-(4-aminophenyl) benzothiazole (**B**), 1*H*-pyrrolo[2,3-*b*]pyridine (**C**), 1,3,5-triazine (**D**) and phenanthridine (**E**) (**Figure 1.12**). We synthesized the analogs and evaluated their anticancer activity by using MTT assay, cell proliferation assay and SRB assay, along with *in vitro* studies to understand the interaction and binding of the synthesized novel compounds, molecular docking was performed using GLIDE-SP program. Molecular docking studies revealed that all the synthesized compounds bind to colchicine, amsacrine and crizotinib. Docking scores were calculated. For the most active compounds the docking pose and their interaction with amino acids is presented.

UV-Vis absorption spectroscopy is the simplest and most commonly employed instrumental technique for studying DNA interactions with small molecules. The study of synthesized active molecule–DNA interactions is carried out by UV-Visible absorption spectroscopy through monitoring changes in the absorption properties of the synthesized molecule or the DNA. Fluorescence spectroscopy is a commonly used technique in the study of interactions between small molecules and DNA due to its high sensitivity. If the molecule shows binding with DNA in absorption spectroscopy, it is confirmed with fluorescence spectroscopy and quenching constant is calculated with stern volmer equation.

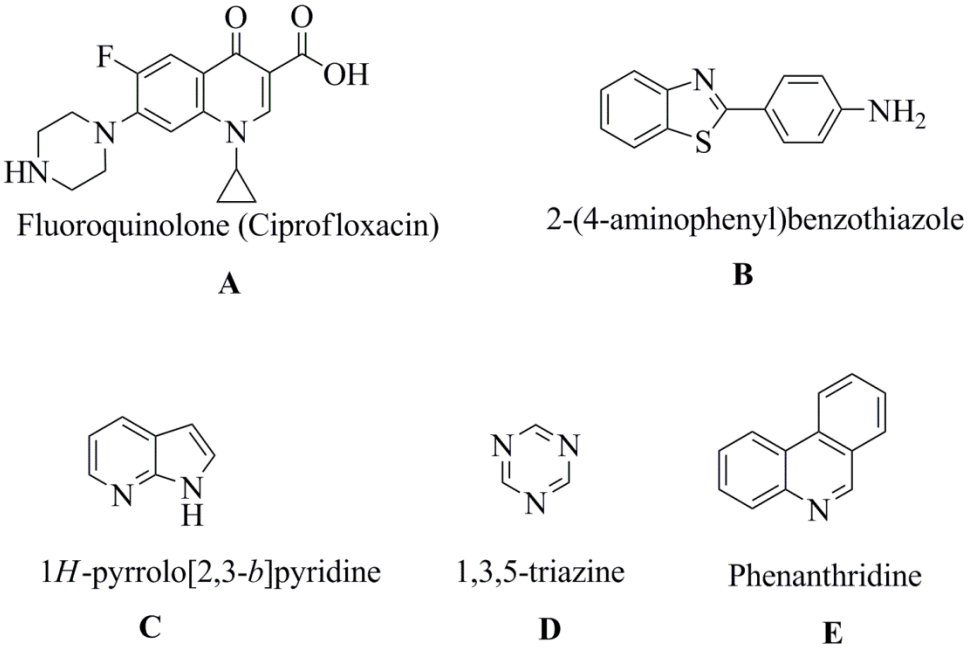

Figure 1.12: Structures of selected heterocyclic scaffolds

Literature review of fluoroquinolone, 2-(4-aminophenyl)benzothiazole, *1H*-pyrrolo[2,3-*b*]pyridine, 1,3,5-triazine and phenanthridine analogues and their antiproliferative activity is discussed in next chapters.

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Chapter II

Objectives

Objectives

Chapter 2

After thoroughly literature review of existing and new promising anticancer drugs, we concluded that lot more work can be done in developing enhance anticancer agents having better qualities over the existing ones in terms of potency.

Hence the main objectives of the proposed research are as follows:

1. To design molecules based on reported anticancer leads.

2. To synthesize the designed novel anticancer molecules.

3. To undertake *in vitro* anticancer screening of the synthesized compounds against various human cancer cell lines.

4. Find out amino acid interactions with molecular docking studies and calculated the standard precision scores of synthesized compounds.

5. To study the interaction between synthesized molecule and DNA with absorption spectroscopy and fluorescence spectroscopic techniques and calculating binding constant.

Chapter III

Synthesis of 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro quinoline-3-carboxylic acid analogues as anticancer agents

Chapter 3

Synthesis of 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro quinoline-3-carboxylic acid analogues as anticancer agents

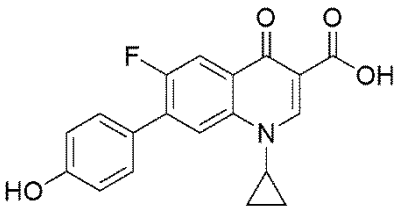
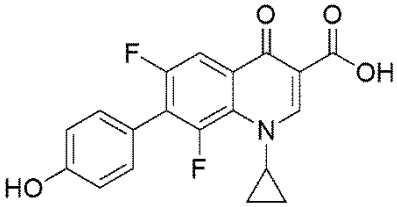
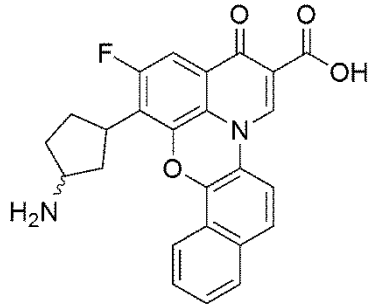
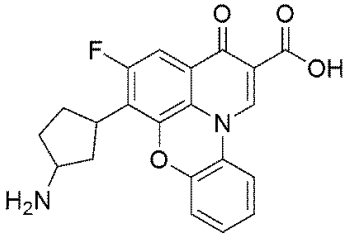
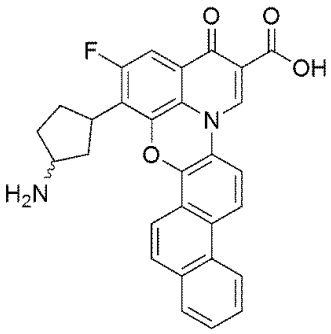
3.1. Introduction

1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro quinoline-3-carboxylic acid [ciprofloxacin (CP)] is one of the broad-spectrum fluoroquinolone (FQ) antibiotics with low side effects [1]. CP exhibited antiproliferative and apoptotic activities in several cancer cell lines such as hormone resistant prostate cancer cell line (PC-3) [2], transitional cell carcinoma cell lines (MBT-2 and T24) [3], colon carcinoma cell lines (CC-531,SW-403 and HT-29) [4], human lymphoidal cell lines (Jurkat) [5], non-small-cell lung cancer cell lines (NCI-H460 and A549) [6,7], ovarian cancer cell line (CHO AA8) [8], murine glioma cell line (GL26) [9], bladder cell line (HTB9). CP was also found to exhibit cell cycle arrest at the S/G_2-M checkpoints [10].

Along with CP, other FQ derivatives like ofloxacin, levofloxacin, enoxacin and fleroxacin were shown to inhibit the growth of transitional cell and bladder carcinoma cell lines [1]. CP enhanced the antiproliferative effect of 5-fluorouracil [11], used for treatment of colon cancer [12]. FQs inhibit the activity of mammalian DNA topoisomerases I, II and DNA polymerase enzymes involved in supercoiling, transcription, replication and chromosomal separation of prokaryotic DNA [4, 13].

Many reports indicate, antitumor efficacy of FQs can be augmented by increasing the lipophilicity of compounds [1, 14, 15]. Introducing substituent's at C-7 position of camptothecin improved the lipophilicity and this led to the discovery of gimatecan, which is currently in phase II clinical trials [16]. In *bis*-quinolinium compounds, lipophilicity of the substituent enhanced their antiproliferative activity against HT-29 colon cancer cell line [17]. Structure of FQ derivatives as anticancer agents is depicted in **Table 3.1** [18-20].

Table 3.1: Lead structures of some fluoroquinolone anticancer agents

Compound	Cell line	IC_{50} Value (μM)
	CHO ovary	20
	CHO ovary	9
	B16 Melanoma MDA-231 Breast H226 non-small cell lung HT-29 colon	0.20 (S), 0.02 (R) 0.08 (S), 0.005(R) 0.03 (S), 0.01 (R) 0.05 (S), 0.03 (R)
	HT-29 colon MCF-7 breast	0.5 0.7
	MCF-7 breast	1 (S), 0.5 (R)

CP showed antiproliferative and apoptotic activities on prostate cancer cell lines (PC3) but not on non-tumorigenic prostate epithelial cells (MLC8891) [21]. CP can impede the acute and chronic prostate inflammation which is responsible for prostate cancer development [22]. The piperazines-based research has attracted significant attention in recent years. Piperazine and substituted piperazines nuclei had constituted an attractive pharmacological scaffold present in various potent marketed drugs. The incorporation of piperazine is an important synthetic strategy in drug discovery due to its easy modifiability, water solubility, the capacity for the formation of hydrogen bonds and adjustment of molecular physicochemical properties [23]. Piperazine, caused inhibition of proliferation of a wide range of cancer cell lines including a multidrug-resistant cell line, with an average IC_{50} of 85nM [24]. These results once again highlighted that piperazine core was an important backbone and prompted us to design some active molecules with piperazine nucleus. Hence we impelled our research work and synthesized new FQ derivatives as antitumor agents. In this study, we synthesized 7-(substituted piperazin-1-yl) derivatives of CP with acetyl link and different substituted piperazines (**Scheme 1**).

Pigeon *et. al*., synthesized aniline or acetanilide hooked 2-ferrocenyl-1,1-diphenyl-but-l-ene derivatives and demonstrated that aniline or acetanilide group enhanced the anticancer activity of the molecule when evaluated against breast cancer cells [25]. Hence, we envisaged to synthesize C7-piperazinyl **CP** acetanilide hybrids anticipating enhanced physicochemical properties of **CP** and/or synergistic effect through combining **CP** and acetanilide in one compact structure (**Scheme 2**).

In recent years, copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction has become a synthetic cornerstone for conjugating building blocks with diverse functionalities [26]. The CuAAC reaction has been widely utilized for synthesizing hybrid molecules exhibiting various biological activities such as in medicinal chemistry [27-30]. This reaction has also been successfully applied in molecular hybridization approach to generate novel hybrid compounds for anticancer activity. Singh *et. al.*, reported 1,2,3-triazole tethered β-lactam-chalcone bifunctional hybrids as anticancer agents [31], Duan *et. al*., synthesized 1,2,3-triazole-dithiocarbamate hybrids [32] as well as 1,2,3-triazole-dithiocarbamate-urea hybrids [33] and evaluated them for the anticancer activity against selected human tumor cell lines. Some of the

compounds exhibited excellent broad spectrum anticancer activity. Ahmed *et. al*., synthesized flavone-triazole-tetrahydropyran conjugates and evaluated the compounds for anticancer activity, in which most of the compounds exhibited IC_{50} in the range of 0.61-1.68 µM [34]. Ma *et. al*., synthesized 1,2,3-triazole-pyrimidine hybrids, which showed IC_{50} values ranging from 1.42 to 6.52 µM against various cancer cell lines [35].

The study of novel hybrid system of 1,2,3-triazole and FQs is not yet explored in anticancer research field. In continuation of our work on CP, we designed and synthesized compounds by combining the CP with 1,2,3-triazole analogues using molecular hybrid approach (**Scheme 3**). Fruitful anticancer results of CP derivatives have attracted us to investigate new lipophilic derivatives of CP as antitumor agents. Hence, we impelled our research work and synthesized new FQ derivatives as antitumor agents.

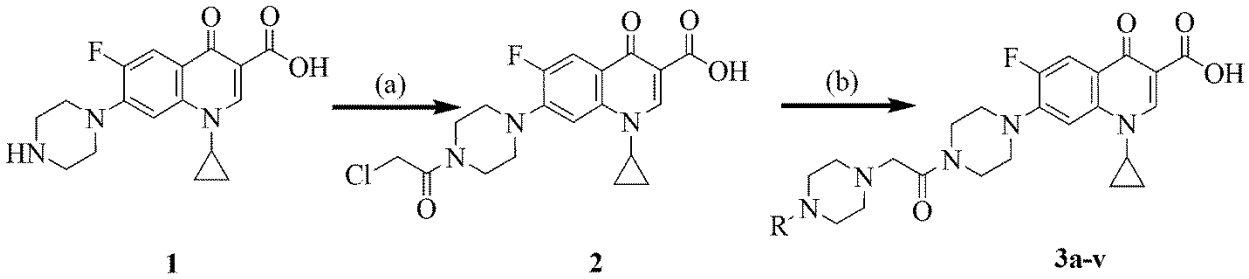
3.2. Results and Discussion

3.2.1. Chemistry

Scheme 1: ***Synthesis of 1-cyclopropyl-6-fluoro-7-(4-(2-(4-substituted piperazin-1-yl) acetyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid analogues as anticancer agents***

We synthesized twenty two novel 7-(substituted piperazin-1-yl) derivatives of CP. Firstly, 7-(4-(2-chloroacetyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2**) was synthesized by coupling CP with chloroacetyl chloride as reported earlier [1] and then various substituted piperazines were reacted with **2** to enhance the lipophilicity.

Acylation of **CP, 1** with chloroacetyl chloride in CH_2Cl_2 yielded compound **2** in 70% after purification. The series of 1-cyclopropyl-6-fluoro-4-oxo-7-(4-substituted piperazin-1-yl)-1,4-dihydro quinoline-3-carboxylic acid derivatives of **CP** were prepared by coupling commercially available substituted piperazines with **2** in *N,N*-dimethylformamide (DMF) to obtain the final compounds **3a-v** in yields ranging from 55- 95%. (**Scheme 1**, **Table 3.2**)



R= methyl, ethyl, acetyl, phenyl, benzyl, 2-Pyridyl, 2-pyramidyl, 2-ClC_6H_4 , 2-CNC_6H_4 , 3-$OCH_3C_6H_4$, 3-$CH_3C_6H_4$, 3-OHC_6H_4, 3-$CF_3C_6H_4$,4-ClC_6H_4 , 4-CNC_6H_4 , 4-$NO_2C_6H_4$, 3,4-di-$OCH_3C_6H_3$, 3,4,di-F-C_6H_3

Scheme 1: Synthetic protocol utilized for the synthesis of compounds **3a-v**

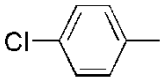
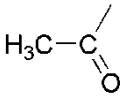
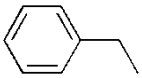
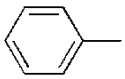
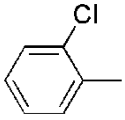
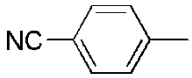
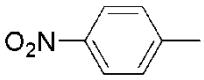
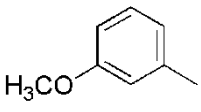
Reagents and conditions: (a) Et_3N, $ClCH_2COCl$, CH_2Cl_2, 0 °C-RT, 1h.

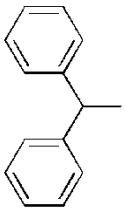
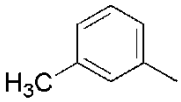
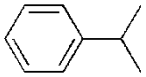
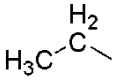
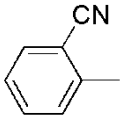
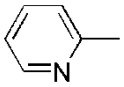
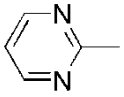
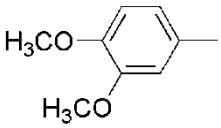
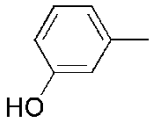
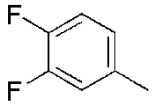
(b) Et_3N, KI, aromatic and aliphatic substituted piperazines, 125 °C, 8-12h.

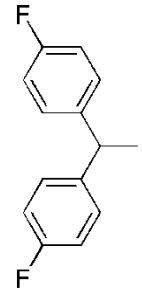
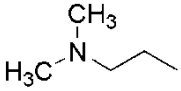
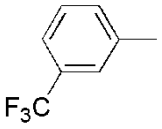
In general ^1H NMR of all the title compounds displayed two triplets in the range 1.10-1.37 ppm and a multiplet in the range 3.75–3.90ppm corresponding to the protons of cyclopropyl ring. Two multiplets of piperazine protons resonated in the range 3.30-3.70 ppm. Two sharp doublets resonated in the range 7.20-7.90 ppm due to C-5 and C-8 protons of the FQ moiety. The C-2 protons of FQ showed a sharp singlet in the range 8.63-8.71 ppm.

A broad peak due to the proton of carboxylic acid functional group resonated in the range 15.15-15.25 ppm. The acetyl link protons showed multiplet in the range 3.29-3.39 ppm and second piperazine protons resonated in the range of 2.20-2.60 ppm.

Table 3.2: Synthesized compounds: structure, yield, and lipophilicity **(3a-v)**.

Entry	R	Yield (%)	clogP[a]
3a		57	-1.20
3b		68	2.54
3c		88	-0.81
3d		89	1.95
3e		88	1.66
3f		95	2.54
3g		68	1.49
3h		92	1.77
3i		87	1.61

3j		82	3.30
3k		65	2.09
3l		94	2.19
3m		94	-0.67
3n		63	1.49
3o		81	0.71
3p		91	-0.05
3q		69	1.27
3r		79	0.02
3s		72	2.10

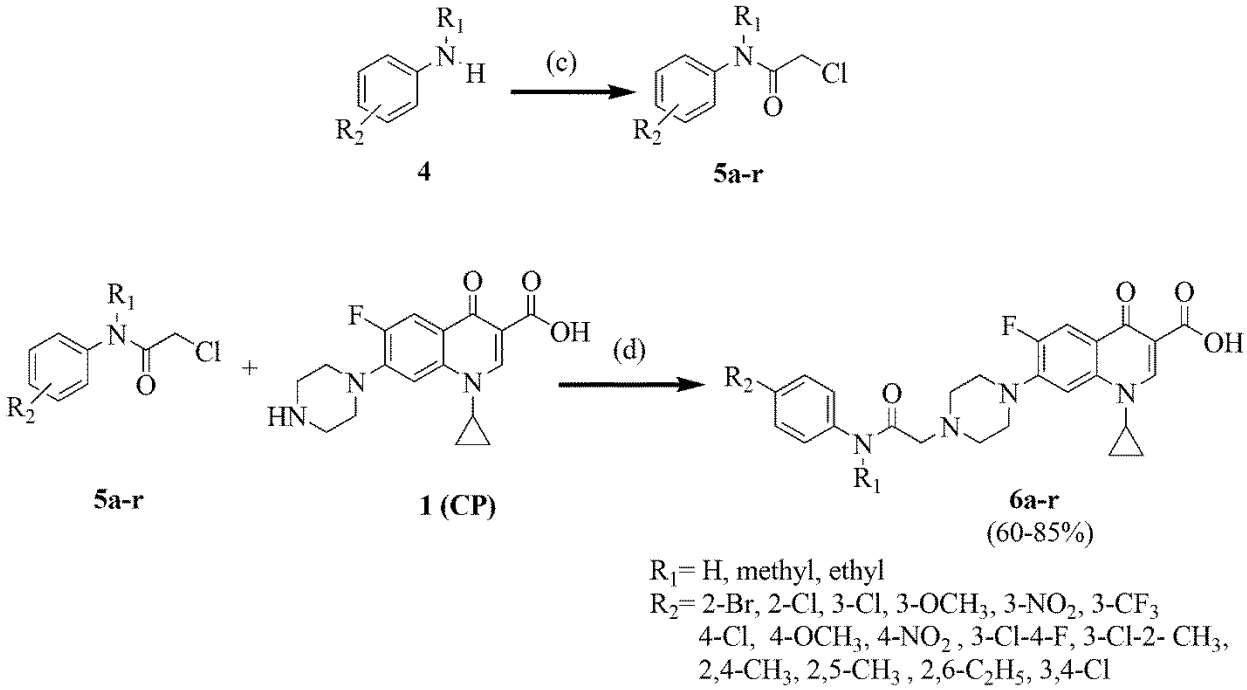
3t		66	3.58
3u		75	-0.82
3v		56	2.84
Ciprofloxacin			-0.72

[a] clogP was calculated by software (Chem Draw Ultra 10.0)

Scheme 2: ***1-cyclopropyl-6-fluoro-7-(4-(2-(substituted(substitutedphenyl)amino)-2-oxoethyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid analogues as novel anticancer agents***

We synthesized total eighteen **(6a-r)** 7-(substituted piperazin-1-yl) derivatives of **CP** in two step process. First step includes preparation of 2-chloro-*N*-(substituted phenyl) acetamides (**5a-r**) by coupling substituted anilines with chloroacetyl chloride. 2-Chloroacetyl chloride was slowly added dropwise to a mixture of various anilines and Et_3N in CH_2Cl_2 at 0 °C. The crude product was purified by crystallization using mixture of ether/hexane (**5a-r**).

In the second step, 2-chloro-*N*-(substituted phenyl) acetamides were coupled with **CP** yielding the title compounds. To a solution of **CP** in dry DMF, triethylamine and potassium iodide were added at RT under N_2 atmosphere. To the resultant mixture, **5a-r** was added and heated at 125 °C. The resultant crude was purified by column chromatography [CH_2Cl_2/MeOH (10%)] to get the title compounds (**6a-r**). (**Scheme 2**, **Table 3.3**).



Scheme 2: Synthetic protocol utilized for the synthesis of compounds **6a-r**

Reagents and conditions: (c) 2-chloroacetyl chloride, Et_3N, CH_2Cl_2, 0-25 °C, 20 h

(d) Et_3N, KI, DMF, 125 °C, 12h

Table 3.3: Synthesized compounds: structure, M.P, yield and docking score (**6a-r**)

Entry	R_2	R_1	M.P (°C)	Yield (%)	Docking Score (SP)
6a	4-Cl	H	222-224	73	-7.963
6b	3-Cl	H	249-251	67	-7.743
6c	2-Cl	H	289-290	63	-7.78
6d	3-OCH_3	H	219-221	77	-7.694
6e	4-OCH_3	H	208-210	67	-7.95
6f	3-Cl-4-F	H	245-246	64	-7.848
6g	2-Br	H	284-285	65	-7.892

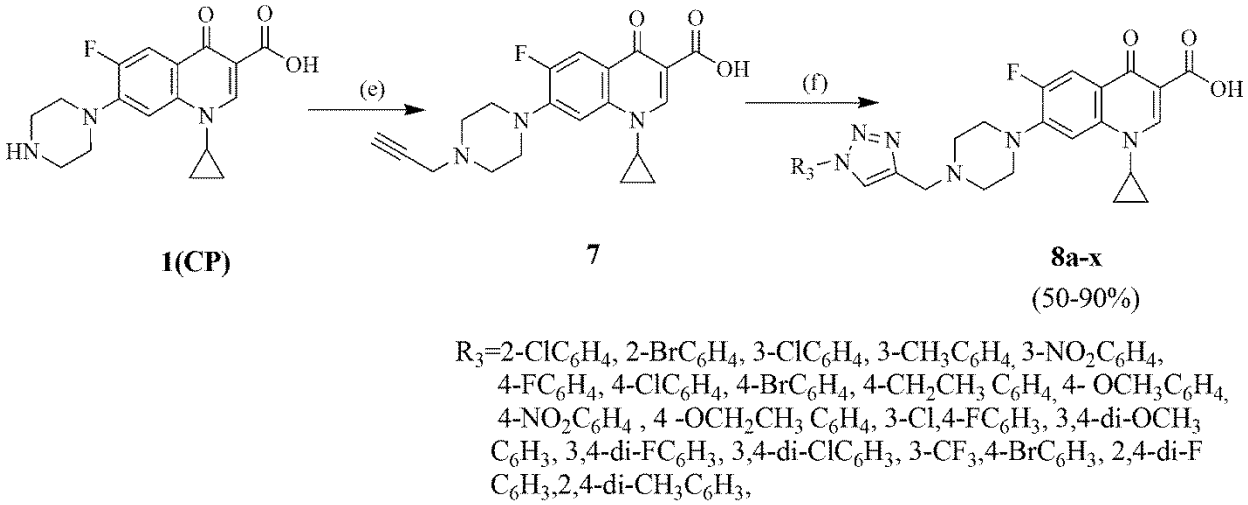
6h	H	H	259-261	82	-7.831
6i	H	CH_3	189-190	85	-7.206
6j	3-NO_2	H	265-266	82	-7.521
6k	H	CH_2CH_3	180-182	64	-5.382
6l	4-NO_2	H	257-258	60	-7.924
6m	3-CF_3	H	222-223	65	-8.048
6n	3-Cl-2-CH_3	H	268-269	63	-7.847
6o	2,4-diCH_3	H	256-258	65	-7.446
6p	2,5-diCH_3	H	244-245	62	-7.68
6q	2,6-diC_2H_5	H	261-262	67	-7.459
6r	3,4-diCl	H	214-216	71	-7.729
CP					-7.577

Scheme 3: ***1-cyclopropyl-7-(4-((1-substituted-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro -4-oxo-1,4-dihydroquinoline-3-carboxylic acid analogues as anticancer agents***

Alkylation of **CP** with propargyl bromide in *N*,*N*-dimethylformamide yielded compound **7** in 80% after column purification with dichloromethane and methanol (10%). In second step, we synthesized 1-cyclopropyl-6-fluoro-4-oxo-7-(4-((1-substituted-1H-1,2,3-triazol-4-yl)methyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid, the title compounds, *via* click chemistry approach utilizing azide-alkyne cycloaddition reaction.

To a stirred solution of compound **7** and substituted phenyl azide in tertiary butanol-water (1:1), $CuSO_4.5H_2O$ and sodium ascorbate were added and the reaction mixture was stirred at RT. (**Scheme 3**, **Table 3.4**). After completion of the reaction, as indicated by TLC, butanol was removed under reduced pressure. The residue was extracted with chloroform (3 x10 mL) and combined organic layers were collected and washed with saturated brine solution, dried over anhydrous $MgSO_4$ and concentrated in vacuo to get the crude product. The product was further

purified by column chromatography using dichloromethane and methanol (10%) to afford the title compounds.

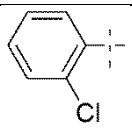
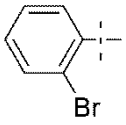
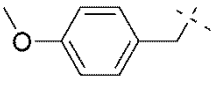


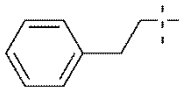
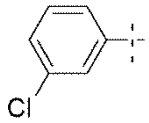
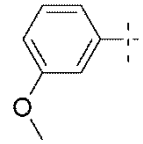
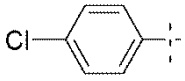
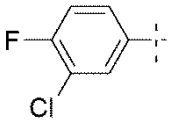
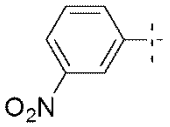
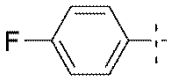
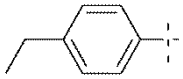
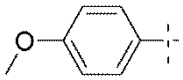
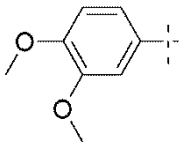
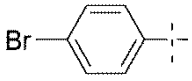
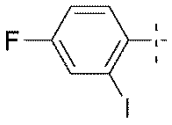
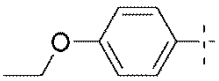
Scheme 3: Synthetic protocol utilized for the synthesis of molecules **8a-x**

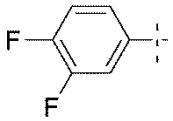
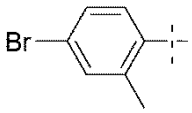
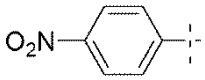
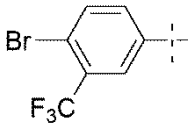
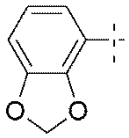
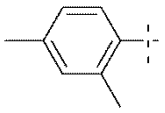
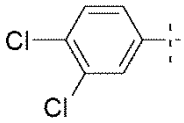
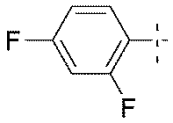
Reagents and conditions: (e) propargyl bromide, K_2CO_3, DMF, 70 °C, 1h

(f) various aromatic azides, $CuSO_4.5H_2O$, sodium ascorbate, *t*BuOH:H_2O (1:1), 2h

Table 3.4: Synthesized compounds: structure, M.P, yield and docking scores (**8a-x)**.

Entry	R_3	M.P (^{0}C)	Yield (%)	Docking Score (SP)
8a		210-212	68	-8.550
8b		228-229	79	-8.437
8c		225-227	71	-7.266

8d		190-191	73	-8.099
8e		100-101	62	-8.167
8f		154-155	61	-8.236
8g		192-193	66	-7.721
8h		118-120	68	-9.248
8i		110-112	90	-8.363
8j		144-145	51	-7.642
8k		165-166	72	-7.956
8l		132-133	50	-7.920
8m		108-110	50	-8.293
8n		199-200	53	-7.484
8o		232-233	60	-9.808
8p		187-188	67	-8.253

8q		118-119	73	-9.345
8r		158-159	50	-8.301
8s		265-266	63	-8.246
8t		146-147	82	-8.448
8u		147-148	61	-8.300
8v		212-213	75	-7.418
8w		175-176	83	-7.908
8x		152-153	72	-9.498
CP				-7.735
Colchicin				-7.659
Doxorubicin				-7.670

3.2.2. Antiproliferative activity

In scheme 1, the synthesized compounds were evaluated for their inhibitory activity on the proliferation of three cancer cell lines *viz* human caucasian acute lymphoblastic leukemia cells (CCRF-CEM), breast adenocarcinoma cells (MDA-MB-468) and human colon carcinoma cells (HCT-116) by cell proliferation assay. Dox and DMSO were used as positive and negative controls, respectively. The results for the antiproliferative activity of the synthesized CP analogues **3a-v** at 50 µM after 72 h incubation are shown in **Figure 3.1**. Most of the synthesized

CP analogues were not as effective as positive control in inhibiting the proliferation of these cell lines after 72 h incubation. Among all the CP analogues, **3j** inhibited proliferation of MDA-MB-468 up to 35% and compounds **3s** and **3t** with fluoro substituent inhibited proliferation of all three cancer cell lines in the range of 35-60%. **3t** at 50 µM showed comparable potency to Dox (10 µmol) in all three cell lines.

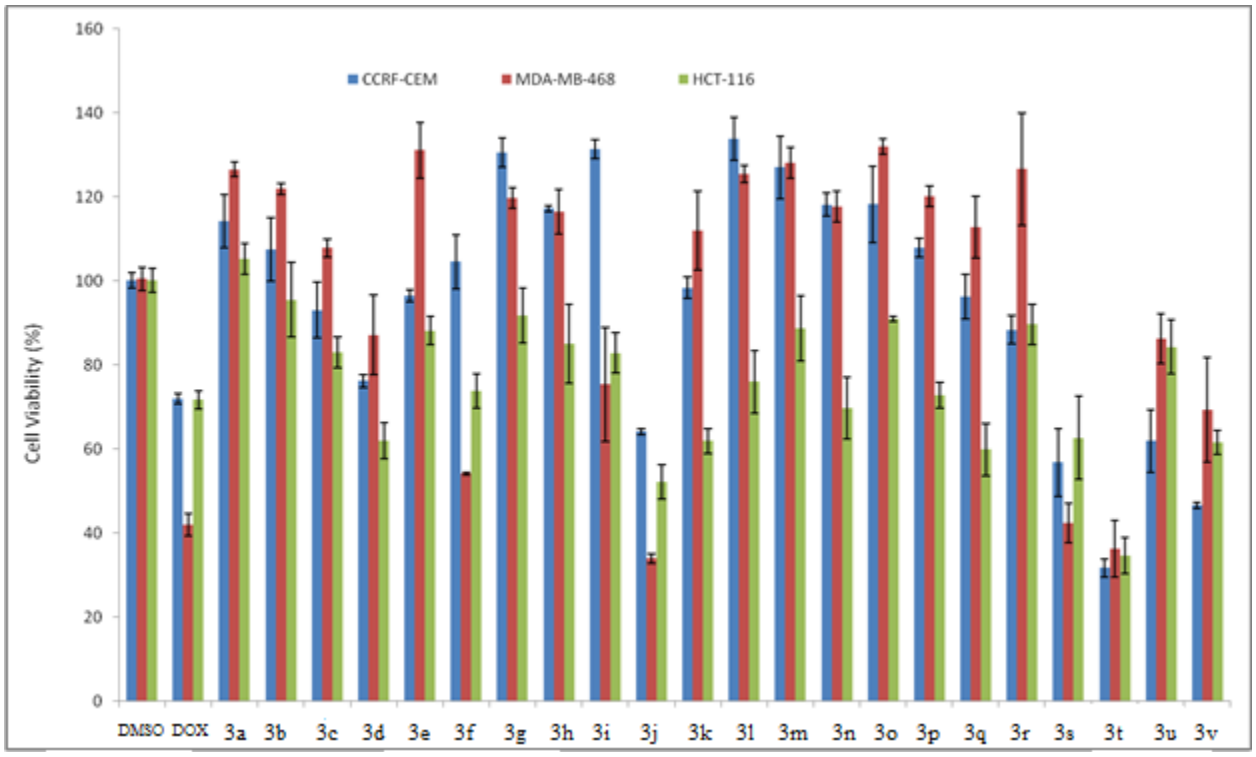


Figure 3.1: Antiproliferative activity of compound **3a-v**

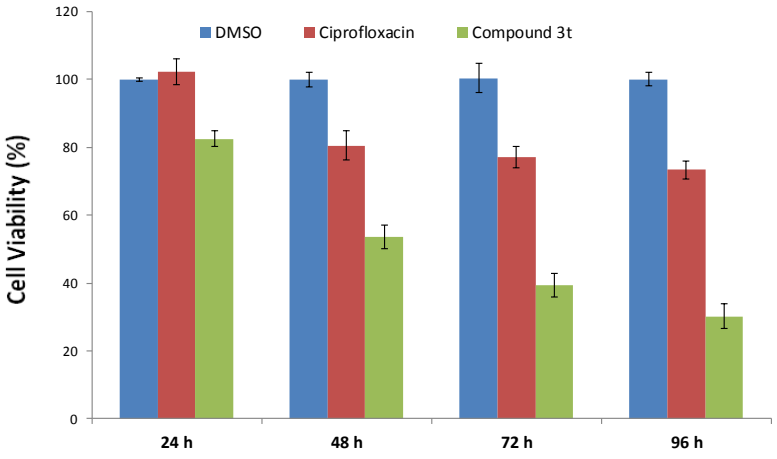


Figure 3.2: Antiproliferative Activity of compound **3t** compared to **CP** in CCRF-CEM cells.

Antiproliferative activity of compound **3t** (50 μM) was compared with that of CP (50 μM) in CCRF-CEM cells in a time dependent manner (24-96 h). Derivative **3t** showed higher inhibitory potency than that of CP. The effect of the compound was found to be time dependent as cell proliferation inhibitory activity of the compound **3t** was enhanced at longer incubation period with cells. For example, derivative **3t** showed 19%, 27%, 38%, and 43% higher antiproliferative activity when compared with that of CP after 24, 48, 72, and 96 h incubation (**Figure 3.2**).

In scheme 2, we concentrated on the synthesis, antiproliferative evaluation and DNA binding. The synthesized compounds **(6a-r)** were evaluated for their *in vitro* antiproliferative activity on five human cancer cell lines by MTT assay (**Table 3.5**). There is curiosity in discovering the binding of molecules with DNA for the rational design and construction of efficient drugs. In the current study we evaluated the DNA-binding interactions of new synthesized **CP** derivative by using fluorescence spectroscopic technique.

All the compounds **6a-r**, showed significant growth inhibition on A549 cell line with IC_{50} values ranging from 11.69 ± 0.26 to 15.27 ± 1.68 μM. Substitution of chloro, methoxy at various positions (**6a**, **6b**, **6c** and **6d**) demonstrated lowest IC_{50} and better growth inhibition whereas compounds bearing electron withdrawing groups (**6j** and **6l**) like nitro at meta and para position exhibited moderate activity against A549 (lung cancer) cell line. Substitution with 2,4-dimethylphenyl at C7 position (**6o**) exhibited promising anticancer activity against Miapaca, HeLa, MDA MB-231 cancer cell lines. Nevertheless, **6o** was found to be potent than **CP** against MCF7 cell line with IC_{50} value 26.45 μM.

From the IC_{50} values (**Table 3.5**) it is clear that most of the active compounds exhibited less cytotoxicity towards the normal embryonic kidney cell line (HEK) compared to their anti-cancer potential against tested cancer cell lines, which justifies the role of the novel synthesized compounds as anti-cancer agents.

Table 3.5: IC_{50} values (μM) for compounds (**6a–r**) in five human cancer cell lines (A549, MiaPaca, HeLa, MDA MB-231, MCF-7) as well as normal cell line HEK

Entry	A549[a]	MiaPaca[b]	HeLa[c]	MDAMB 231[d]	MCF7[d]	HEK[e]
6a	13.29± 0.44	45.4 ± 0.52	>100	34.3 ± 0.41	>100	72.41 ± 1.69
6b	11.69 ±0.26	37.02 ± 0.47	76.3 ± 0.76	32.79 ± 0.4	>100	73.63 ± 1.08
6c	11.71 ±0.18	35.57 ± 0.48	91.95 ± 0.2	49.77 ± 0.42	>100	>100
6d	12.64± 0.37	70.23 ± 1.69	49.08 ± 0.88	30.76 ± 0.82	>100	>100
6e	12.72± 0.83	36.92 ± 0.74	51.77 ± 0.48	>100	86.37 ± 0.61	>100
6f	12.36± 0.35	33.22 ± 0.32	>100	62.85 ± 1.05	76.8 ± 0.89	>100
6g	13.1 ± 1.78	41.14 ± 0.96	77.89 ± 1.13	>100	>100	64.54 ± 0.66
6h	13.83± 1.61	59.22 ± 0.33	79.76 ± 1.76	78.08 ± 0.31	>100	>100
6i	13.25± 0.27	59.35 ± 1.66	75.18 ± 0.92	>100	>100	>100
6j	12.99± 0.67	69.01 ± 0.99	60.23 ± 0.57	41.53 ± 0.57	>100	>100
6k	12.71± 0.85	30.04 ± 0.9	83.02 ± 1.65	42.05 ± 1.25	>100	>100
6l	13.84± 0.94	40.55 ± 1.94	78.57 ± 1.0	44.93 ± 0.22	75.55 ± 1.25	>100
6m	15.27± 1.68	40.25 ± 1.94	77.54 ± 0.26	54.47 ± 0.62	>100	>100
6n	13.74± 0.84	29.12 ± 0.34	61.57 ± 0.52	33.44 ± 0.44	>100	>100
6o	14.21± 0.66	26.6 ± 0.05	29.28 ± 0.11	25.34 ± 0.25	26.45± 0.33	>100
6p	14.09± 0.89	48.94 ± 0.77	68.62 ± 0.25	45.65 ± 0.86	>100	76.58 ± 1.67
6q	13.88± 0.09	35.39 ± 0.22	>100	70.62 ± 1.47	63.92 ± 0.35	>100
6r	13.32± 0.17	36.93 ± 0.31	85.09 ± 0.46	84.34 ± 2.69	>100	>100
CP	19.31± 0.58	21.62 ± 0.28	65.82 ± 0.74	25.47 ± 0.37	>100	96.72 ± 1.23
Dox	4.45 ± 0.02	4.25 ± 0.03	4.29 ± 0.006	4.16 ± 0.01	4.92 ± 0.01	64.58 ± 1.92

[a] lung cancer, [b] pancreatic cancer, [c] cervical cancer, [d] breast cancer, [e] normal embryonic kidney cells

IC_{50} is concentration at which 50% of cells undergo cytotoxic cell death due to compound treatment. IC_{50} values are indicated as the mean ± SD of three independent experiments

CP = Ciprofloxacin, Dox = Doxorubicin

In scheme 3, the synthesized compounds were evaluated for their antiproliferative activity against ovarian carcinoma cell line (SK-OV-3) and Human T cell lymphoblast cell line (CCRF-CEM) by cell proliferation assay. Doxorubicin (Dox) and DMSO were used as positive and negative controls, respectively. The results for the antiproliferative activity of the synthesized **CP** analogues **8a–x** at 10 µM after 72 h incubation are shown in **Figure 3.3**. Substitution of electron withdrawing group like chloro and bromo at various positions showed good activity against CCRF-CEM cell line. Substitution of bromo at ortho position (compound **8b**) resulted in more active compound than bromo at para (**8n**) position. Compared with ortho chloro compound (**8a**), meta (**8e**), para (**8g**) and dichloro (**8w**) compounds are more active against CCRF-CEM cell line. Substitution with electron donating groups like methoxy (**8l**) and ethoxy (**8p**) at para positions showed moderate activity against CCRF-CEM cell line. Compared with para methoxy (**8l**) and 3,4-dimethoxy (**8m**) compounds, meta methoxy (**8f**) substituted compound exhibited good activity against CCRF-CEM cell line. Compound **8k** with electron donating ethyl group at para position was found to be more active than Dox against SK-OV-3 and CCRF-CEM cell lines. Disubstituted compounds **8o**, **8r** and **8t** were found to be more active than Dox against CCRF-CEM.

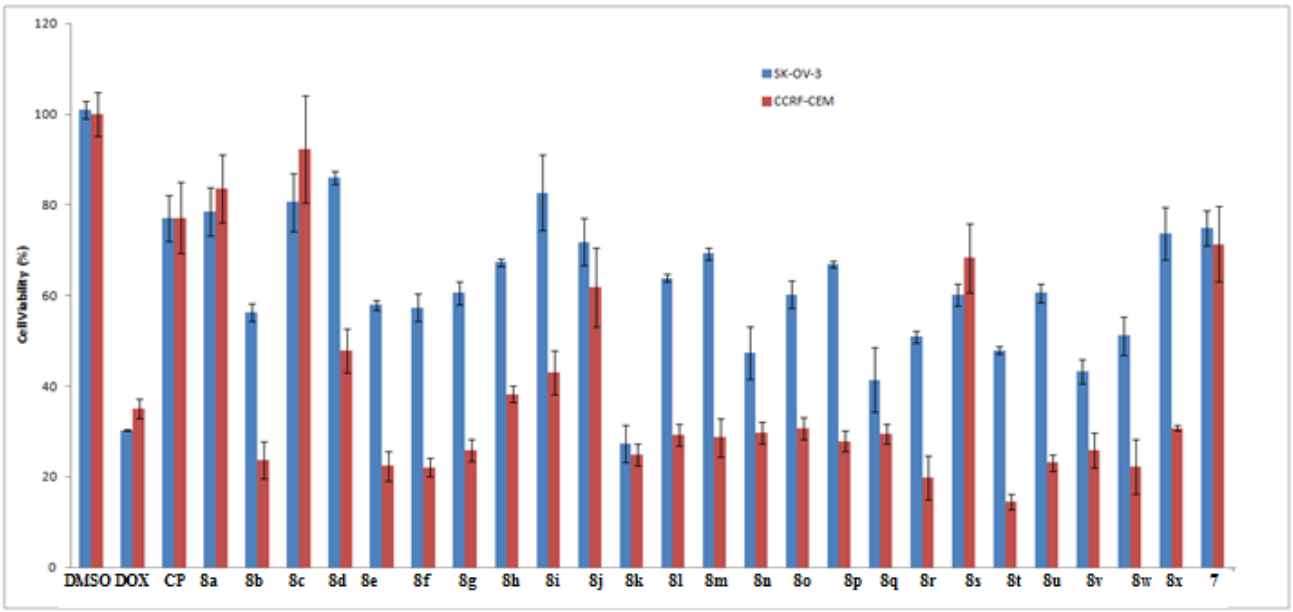


Figure 3.3: Antiproliferative activity of compounds **8a-x**

3.2.3. Molecular docking studies

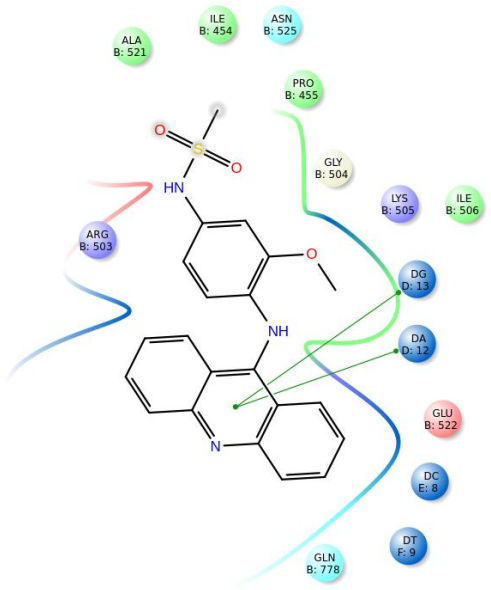
Further the molecular docking studies of **6a-r** were performed using human topo-II isomerase being the target enzyme of **CP** using Schrödinger suite 2013. Crystal co-ordinates for DNA topo-II isomerase was taken from Protein Data Bank (PDB ID: 4G0U). The multi-step Schrödinger's protein preparation tool (PPrep) has been used for final preparation of receptor model.

Hydrogens were added to the model automatically via the maestro interface. PPrep neutralizes side chains and residues which are not involved in salt bridges. This step is then followed by restrained minimization using the OPLS 2005 force field to RMSD of 0.3 A^0.

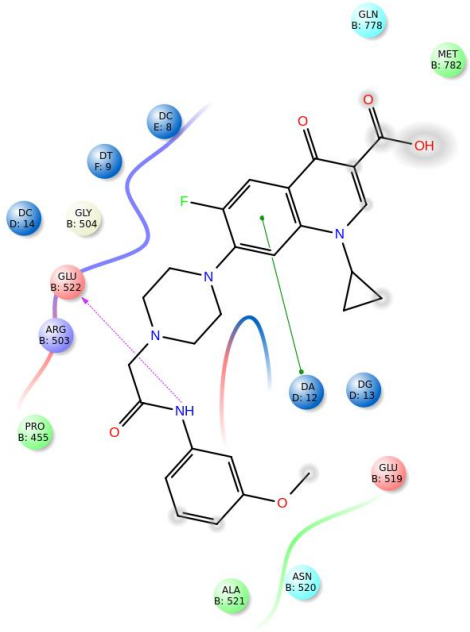
The 2D structure of **6a-r** were sketched and converted to 3D using maestro interface. Ligands were prepared for docking using Ligprep, module of Schrodinger. A total of 10 conformations were generated for all the compounds. Grid box was generated with co-ordinates of X:26.2195, Y:99.8115, Z:32.8506 by considering co-crystal ligand i.e. amsacrine. Docking studies were performed using GLIDE, module of Schrödinger. Docking scores by standard precision (Glide-SP) docking were shown in **Table 3.2**.

Amino acid interaction pattern of few active compounds **6d, 6n**, and **6o** are shown in **Figure 3.4** along with amsacrine (topoisomerase inhibitor) as standard. Amsacrine has shown docking score of -5.86.

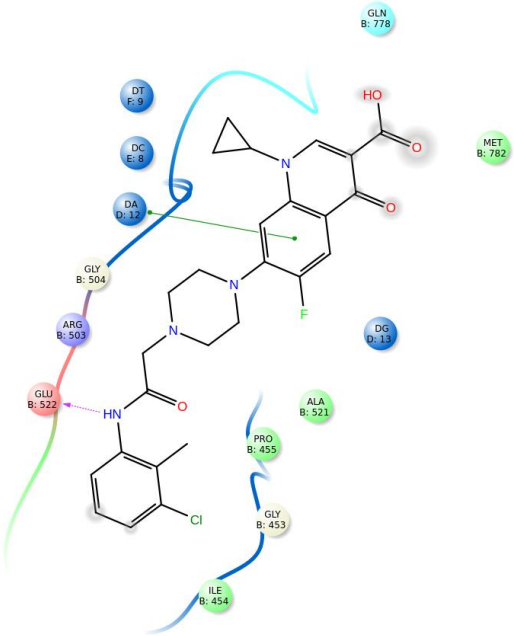
Compounds **6d, 6n**, and **6o** having hydrogen bonding interaction between GLU 522 and amide proton, π-π stacking interactions between fluoroquinolone phenyl ring and DA 12 and DG 13. These interactions might be increased the antiproliferative activity of the synthesized compounds.



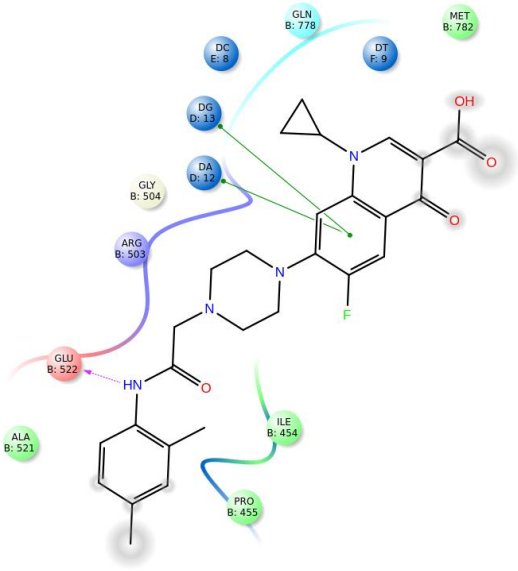
A: Crystal Ligand (4G0U_amsacrine)



B: Compound 6d

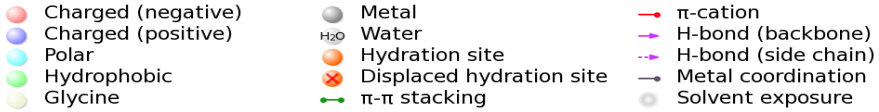


C: Compound 6n

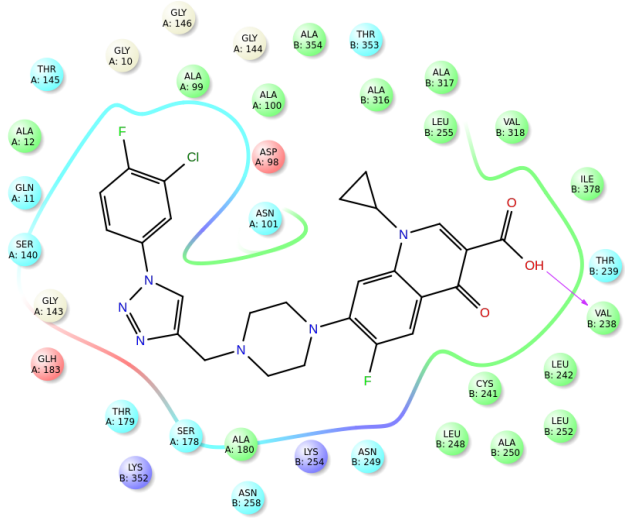


D: Compound 6o

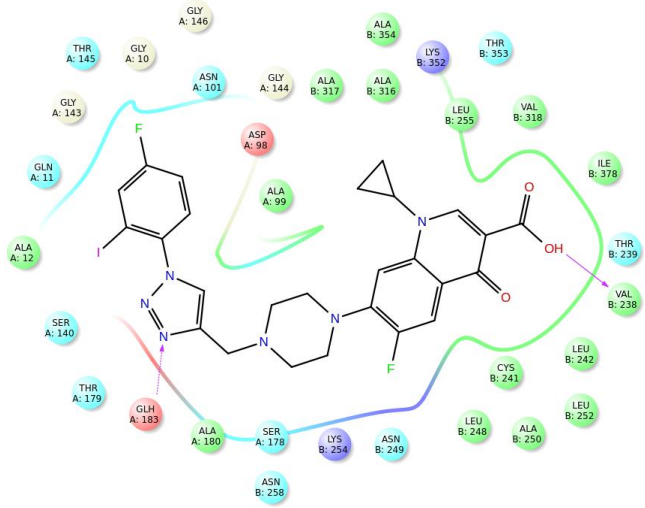
Figure 3.4: Amino acid interaction pattern of compounds **6d**, **6n** and **6o**



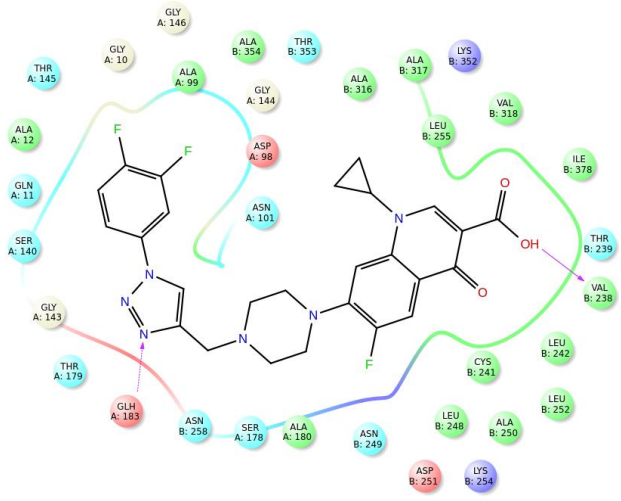
For scheme 3 synthesized compounds, to understand the interaction and binding of the compounds molecular docking was performed using GLIDE-SP program. Molecular docking studies revealed that all the synthesized compounds (**8a-x)** bind to colchicine binding site of tubulin with binding affinity ranging from -9.808 to -7.266 compared to -7.659 of the standard colchicine. Docking scores are displayed in **Table 3.3**. Docking pose and interacting amino acids for the most active compounds **8h**, **8o**, **8q**, **8x** and standard colchicine are shown in **Figure 3.5**.



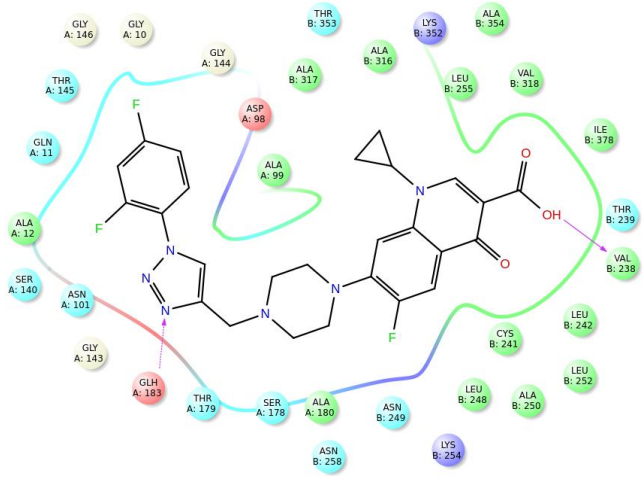
Compound **8h**



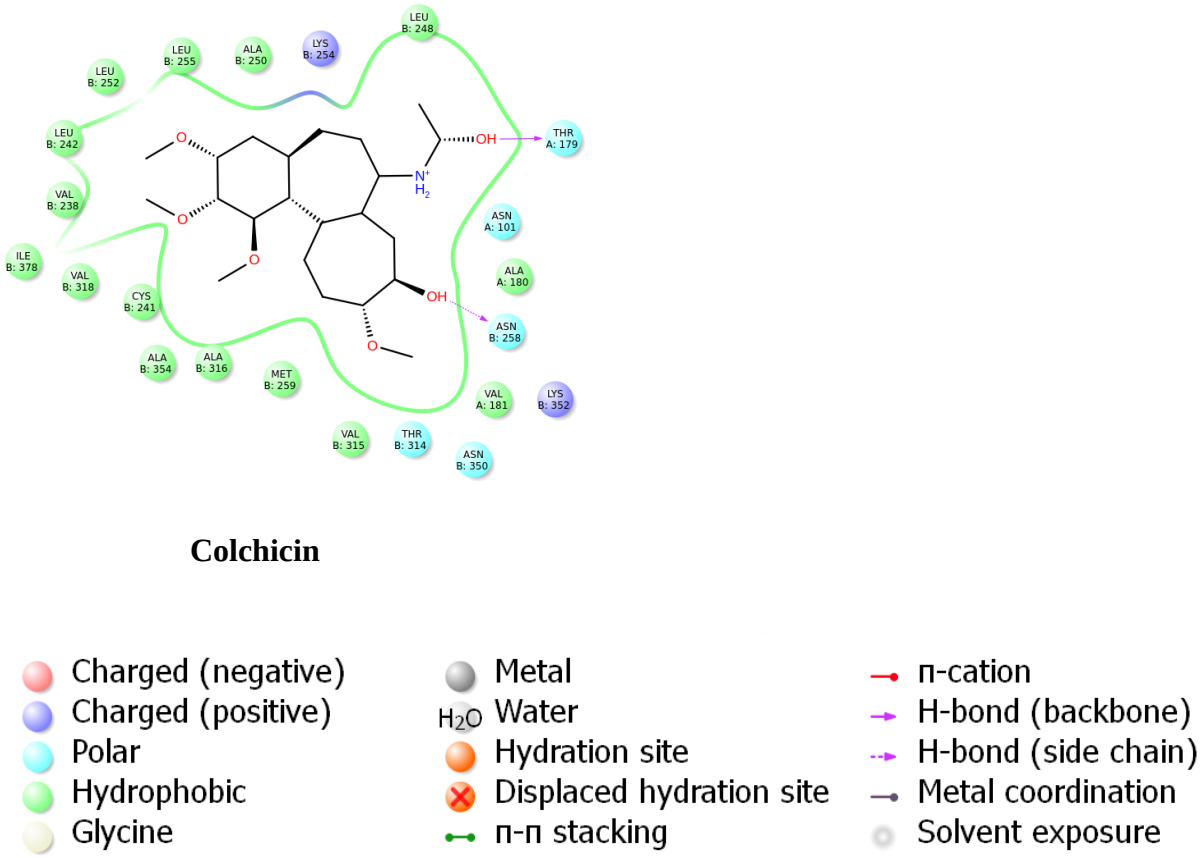
Compound **8o**



Compound **8q**



Compound **8x**



Colchicin

Figure 3.5: Docking pose and interacting amino acids of compounds **8h**, **8o**, **8q**, **8x** and Colchicin.

3.2.4. DNA binding affinity

DNA binding affinity between synthesized compound (**6o**) and CtDNA was studied with UV-visible and fluorescence spectroscopes.

3.2.4.1. UV- Visible spectra studies

UV–visible spectroscopy is regularly used to discover the interaction studies between biological macromolecules and small molecules. We used this technique to investigate the absorbance spectra of **6o**-CtDNA interaction (**Figure 3.6**). The characteristic peak of compound **6o** alone was observed at near 220nm. However, on subsequent addition of CtDNA to compound **6o**, the absorbance of compound was gradually decreasing, that means hypochromic effect.

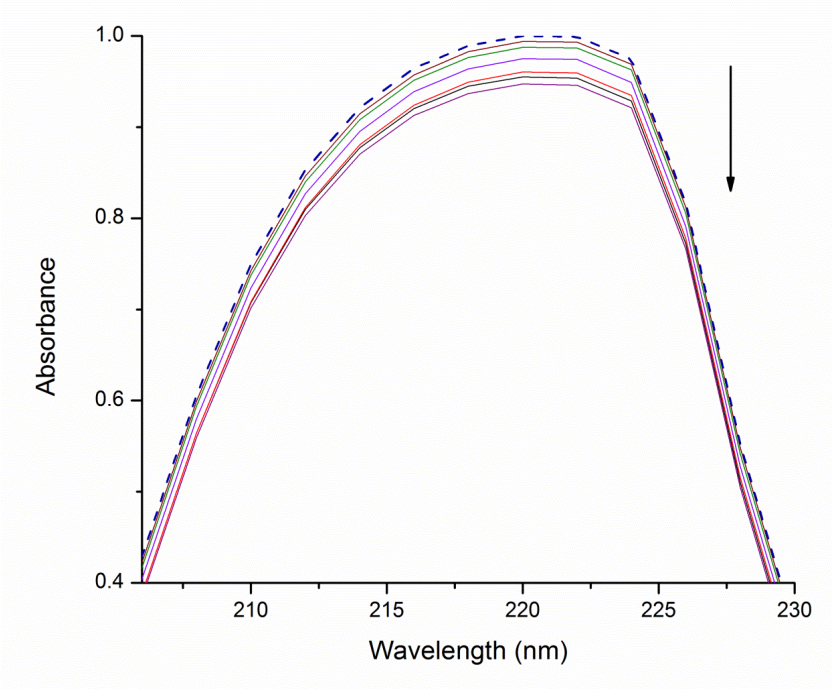


Figure 3.6: The Absorption spectra of Compound **6o-CtDNA** system: [Compound] = 1.64 × 10^{-5} M. Arrow shows the absorption intensity changes (decreases) upon increasing CtDNA concentration.

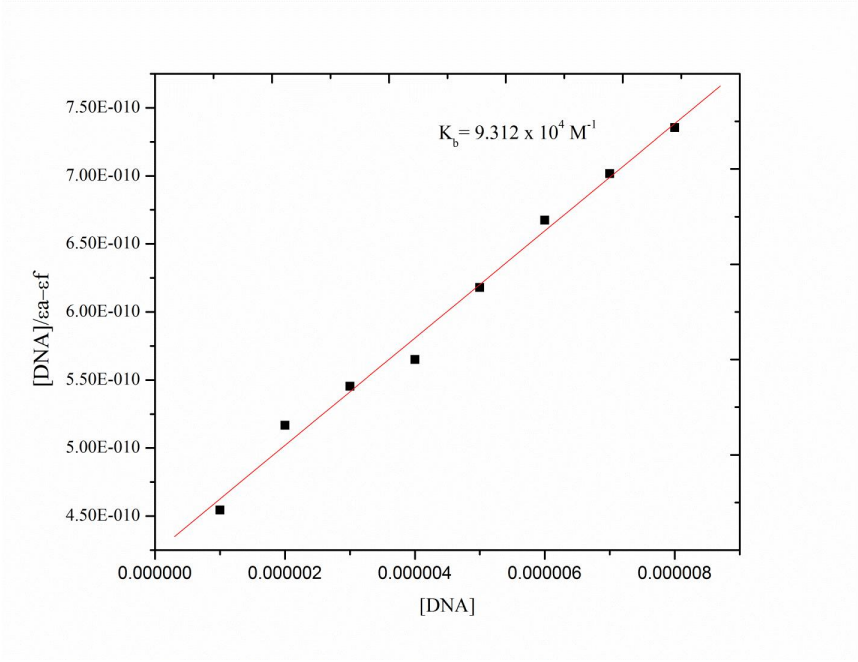


Figure 3.7: Plot of [DNA]/(εa − εf) *vs* [DNA] for the titration of DNA with **6o** compound and solid line is linear fitting of the data.

This hypochromic effect interaction of compound **6o** with CtDNA indicates strong intermolecular interaction. Hypochromic effect is due to the overlap of the electron cloud of the compound **6o** with the CtDNA base pairs [36, 37]. Hypochromic effect in UV–visible spectra upon compound binding to CtDNA is a characteristic of an intercalating binding mode [38, 39].

The intrinsic binding constant K_b of the compound to CtDNA was determined from following equation.

$$[DNA]/|\varepsilon_a - \varepsilon_f| = [DNA]/|\varepsilon_b - \varepsilon_f| + 1/K_b|\varepsilon_b - \varepsilon_f$$

Here [DNA] represents the concentration of DNA in base pairs, and ε_a, ε_f and ε_b are the apparent extinction coefficient (A_{obs}/[M]), the extinction coefficient for free metal complex (M), and the extinction coefficient for the free metal complex (M) in the fully bound form, respectively. K_b is the equilibrium binding constant (in M–1) of complex binding to DNA. In plots of $[DNA]/\varepsilon_a - \varepsilon_f$ Vs [DNA], K_b is obtained by the ratio of slope to intercept (**Figure 3.7**). The binding constant K_b for compound **6o** is 9.312×10^4 M^{-1}. These results indicate that the binding strength of compound **6o** is good through the intercalate mode.

We investigated the absorbance spectra of **8t**-CtDNA interaction. The characteristic peak of compound **8t** alone was observed at near 216 nm. However, on subsequent addition of CtDNA to compound **8t**, the absorbance of compound was gradually decreasing, indicating hypochromic effect (**Figure 3.8**). This hypochromic effect interaction of compound **8t** with CtDNA indicates strong intermolecular interaction.

The binding constant K_b for compound **8t** is 4.516×10^4 M^{-1} (**Figure 3.9**). These results indicate that the compound **8t** also binds to CtDNA through the intercalate mode.

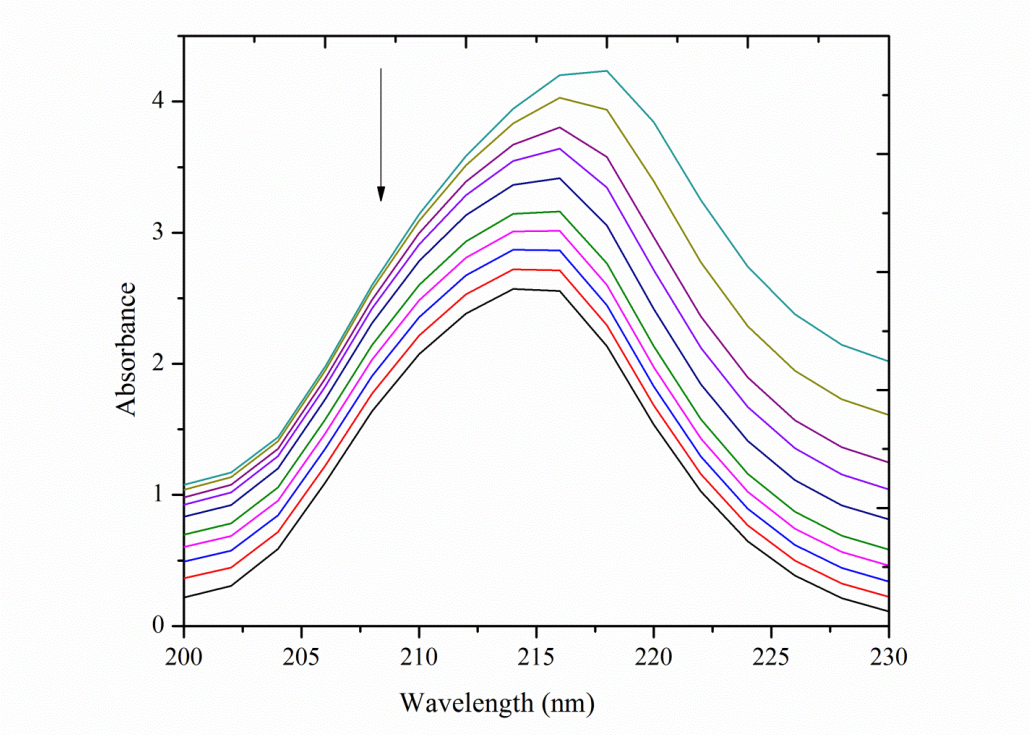


Figure 3.8: The Absorption spectra of Compound **8t**-CtDNA system: [Compound] = 0.015× 10^{-5} M. Arrow shows the absorption intensity changes upon increasing CtDNA concentration

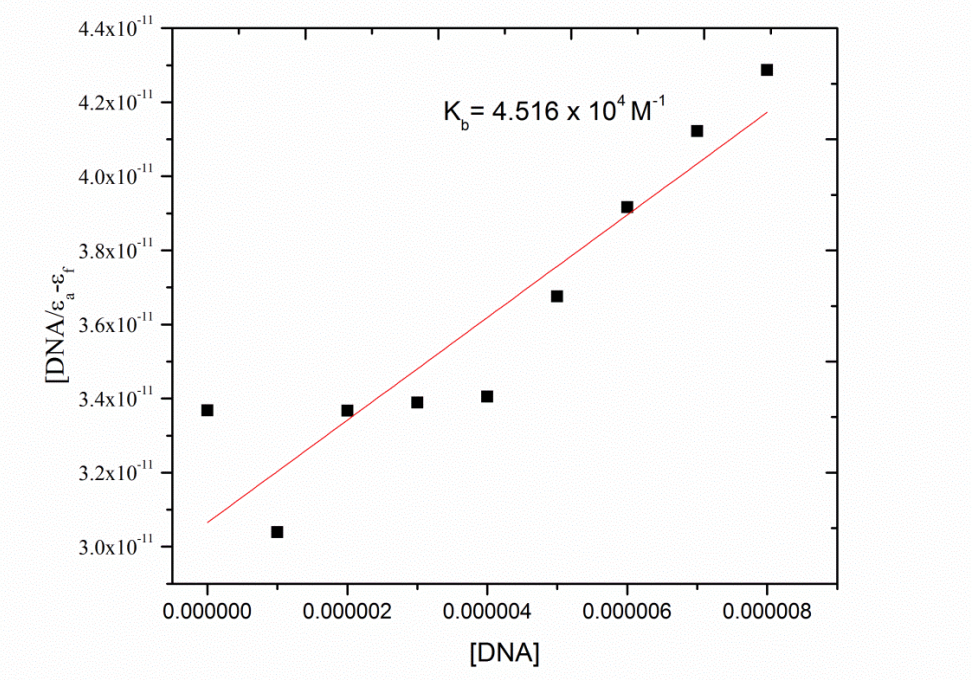


Figure 3.9: Plot of [DNA]/(εa − εf) *vs* [DNA] for the titration of DNA with compound **8t** and solid line is linear fitting of the data

3.2.4.2. Fluorescence spectral studies

EB displays very feeble fluorescence in the aqueous solution, but in the presence of DNA it exhibits strong fluorescence because of the intercalation to base pairs in DNA. Intensity of the EB-DNA adduct allows to determine the affinity of the binding mode of compound (**6o**) for DNA. If compound can replace EB from EB-DNA, the fluorescence of the solution will be quenched owing to the free EB molecules are readily quenched by the adjacent water molecules [40, 41]. The fluorescence quenching of EB-CtDNA by the compound (**6o**) is shown in **Figure 3.10.**

The quenching of EB-CtDNA by the compound **6o** is in good agreement with the linear Stern-Volmer equation, which provides further evidence that **6o** binds to DNA.

$$\frac{I_0}{I} = 1 + K_{sv}\,[Q]$$

In the above equation I_0 is the emission intensity in the absence of quencher, I is the emission intensity in the presence of quencher, K_{sv} is the Stern-Volmer quenching constant, and [Q] is the quencher concentration. The shape of Stern-Volmer plot can be used to characterize the quenching as being predominantly dynamic or static.

Plots of I_0/I versus [Q] appear to be linear. The linear relationship of I_0/Iversus [Q] recommends that the quenching result for this system is a static type, means non-fluorescence complex is formed between compound **6o** and CtDNA. K_{sv} is given by the ratio of the slope to the intercept (**Figure 3.11)**. The K_{sv} value for the compound is $5.1 \times 10^4\ M^{-1}$. This data clearly indicates the interaction of **6o** with CtDNA.

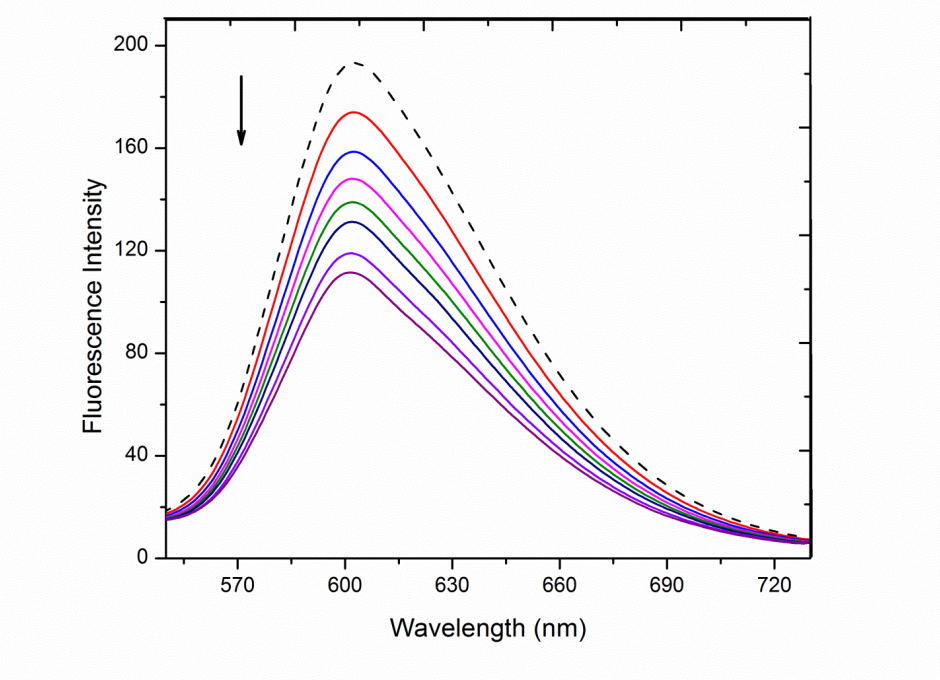


Figure 3.10: The Fluoroscence spectra of DNA-EB system: ex =500nm, em = 520-740 nm, [Compound] = 0-1.64 × 10^{-5} M. CtDNA(------line). Arrow shows the emission intensity changes upon increasing compound (**6o**) concentration.

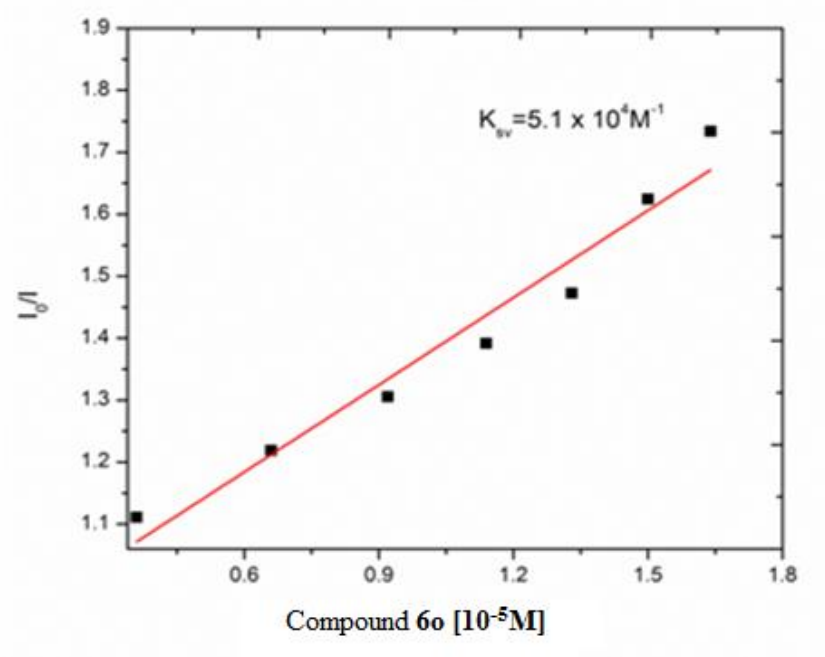


Figure 3.11: Stern-Volmer plot of the fluorescence titration data of the compound (**6o**). (Plots of I_0/I versus [Compound **6o**]).

To further investigate the intercalation mode of the compound **8t** with DNA, the competitive binding experiment using EB as a probe was carried out [40]. EB emits intense fluorescence at about 607 nm in the presence of DNA due to the strong intercalation between the adjacent DNA base pairs. The binding study of compound **8t** and DNA–EB system is shown in **Figure 3.12.** The quenching of EB-CtDNA by the compound **8t** is in good agreement with the linear Stern-Volmer equation, which provides further evidence that **8t**, binds to DNA.

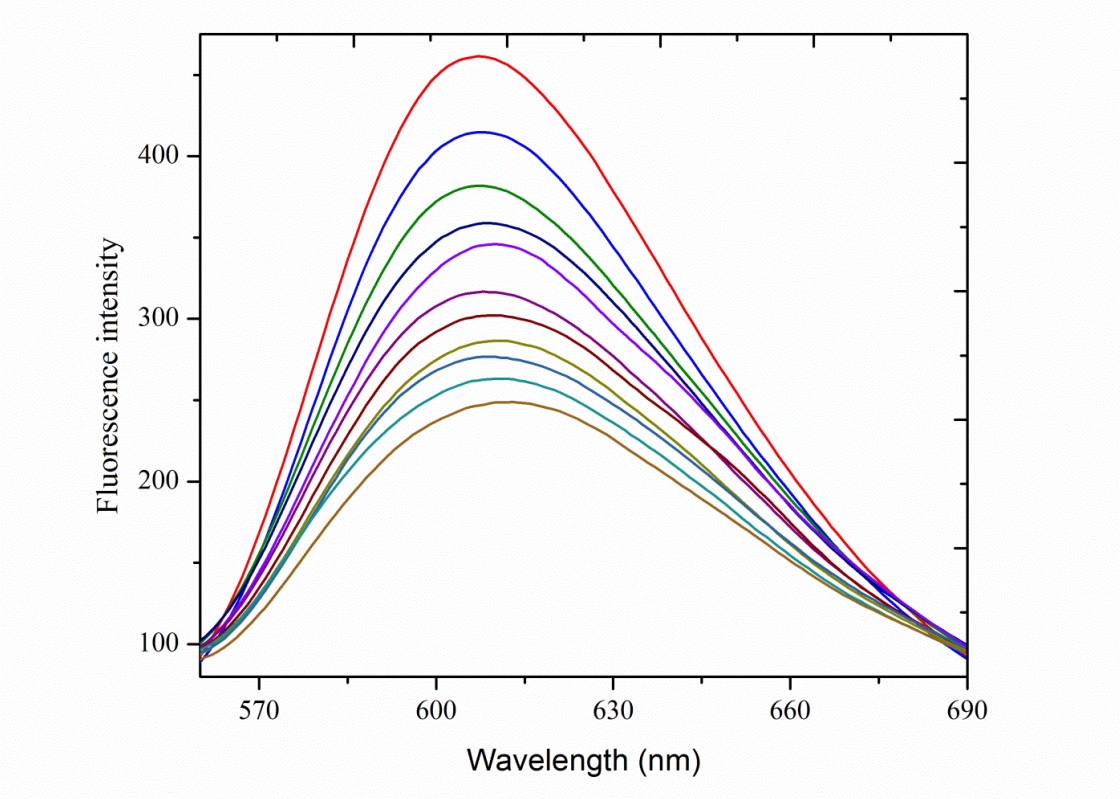


Figure 3.12: The Fluoroscence spectra of DNA-EB system: ex =500nm, em = 520-740 nm, [Compound]= 0-2 × 10^{-5} M. From top to bottom the emission intensity decreases upon increasing compound (**8t**) concentration.

The quenching of EB-CtDNA by the compound **8t** is in good agreement with the linear Stern-Volmer equation, which provides further evidence that **8t,** binds to DNA. Plots of I_0/I versus [Q] appear to be linear. The linear relationship of I_0/Iversus [Q] recommends that the quenching result for this system is a static type, means non-fluorescence complex is formed

between compound **8t** and CtDNA. K_{sv} is given by the ratio of the slope to the intercept (**Figure 3.13)**. The K_{sv} value for the compound is 8.45 × 10^{-2} M^{-1}. This data clearly indicates the interaction of **3t** with CtDNA.

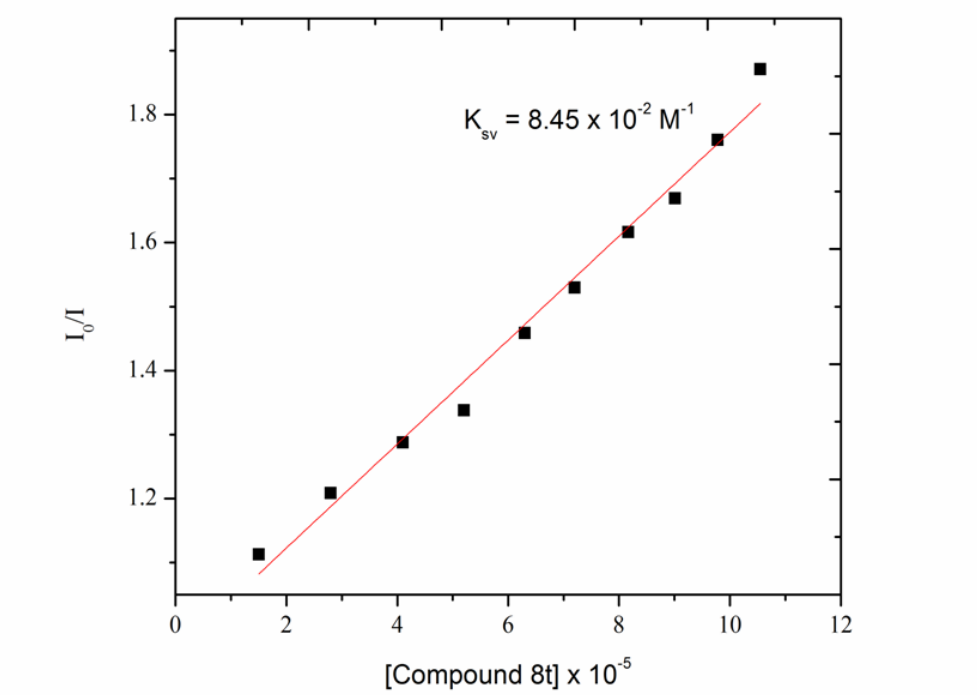


Figure 3.13: Stern-Volmer plot of the fluorescence titration data of the compound (**8t**). (Plots of I_0/I versus [Compound **8t**]).

3.3. Conclusion

In summary, in scheme 1 novel CP analogues were synthesized, emphasizing on lipophilicity and evaluated for their anticancer activity on human caucasian acute lymphoblastic leukemia cells (CCRF-CEM), breast adenocarcinoma cells (MDA-MB-468) and human colon carcinoma cells (HCT-116). Among the synthesized CP analogues, **3t** (fluoro substituent) at 50 µM showed comparable potency to doxorubicin (10 µmol) in all three cell lines, while **3j** (without fluoro substituent) inhibited proliferation of MDA-MB-468 up to 35% selectively over other two cell lines. These results reveal the importance of fluoro substituent and further modification on the chemical structure of CP derivatives could lead to the synthesis of a promising candidate to develop anti-cancer agent.

In summary in scheme 2 we conclude, electron donating substitution affects the antiproliferative activity of **CP** derivatives. These results reveal the importance of chloro, methoxy, methyl substituents at dissimilar positions and further modification on the chemical structure of **CP** derivatives could lead to the synthesis of a promising aspirant to develop potential anti-cancer agent. Many of the synthesized compounds do not exhibit toxic effect on normal human embryonic kidney cell line (HEK) compared with doxorubicin. DNA-binding properties of the synthesized compounds investigated by absorption and fluorescence studies clearly denote that the compound can bind to DNA through intercalation mode.

In summary in scheme 3, twenty four new CP-1,2,3 triazole hybrid analogues were synthesized and evaluated for their antiproliferative activity against ovarian carcinoma cell line (SK-OV-3) and human T cell lymphoblast cell lines (CCRF-CEM). Among all the synthesized compounds, **8b**, **8g**, **8k**, **8r**, **8t** were found to be more active than Dox against CCRF-CEM. Compound **8k** was found to be more active than Dox against SK-OV-3. These results reveal the importance of hybrid approach of FQ – 1,2,3-triazole derivatives and further modifications could lead to a much more promising anticancer agent. A DNA-binding property of the synthesized compound **8t** was investigated by absorption and fluorescence studies clearly denote that the compound **8t** can bind to DNA through intercalation mode.

3.4. Experimental section

3.4.1. Chemistry

All reagents were purchased from commercial available sources and used without further purification. **CP** was purchased from Sigma Aldrich (>98%). A highly polymerized fibrous form of CtDNA and Ethidium Bromide (EB) were purchased from Sigma-Aldrich. All reactions were monitored by analytical Thin Layer Chromatography (TLC) performed on E-Merck 0.25 mm pre coated silica gel glass plates (60 F254). Visualization of the spots on TLC plates was achieved by exposure to UV light. Column chromatography was performed using silica gel (Acme, 100-200mesh). Solvents were dried according to conventional standard methods and purified by distillation prior to use. Melting points were determined using Stuart SMP30 system and are uncorrected. The UV spectral studies were performed on a spectrophotometer (JASCO model V-650). The fluorescence spectra performed on a spectrofluorometer (JASCO model FP-6300). ^{1}H

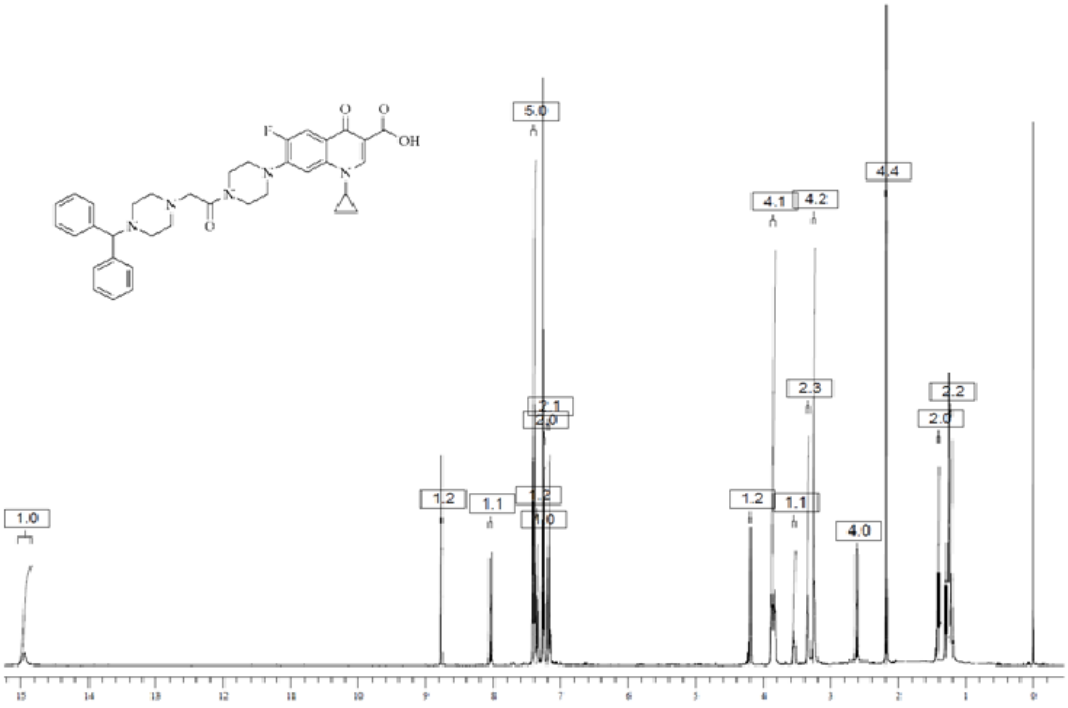
and ^{13}C NMR spectra were recorded on Bruker 400 (400/300 MHz for ^{1}H, 100/75 MHz for ^{13}C), in $CDCl_3$ or DMSO-d_6. Chemical shifts have been expressed in parts per million (δ) relative to tetramethylsilane (δ = 0.0) as an internal standard and coupling constants (*J*) in Hertz. Molecular weights of the synthesized compounds were checked by Shimadzu, LCMS-2020 and the method used was electron spray ionisation (ESI-MS) method.

Synthesis of 7-[4-(2-Chloroacetyl) piperazin-1-yl]-1-cyclopropyl-6- fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ***(2)***

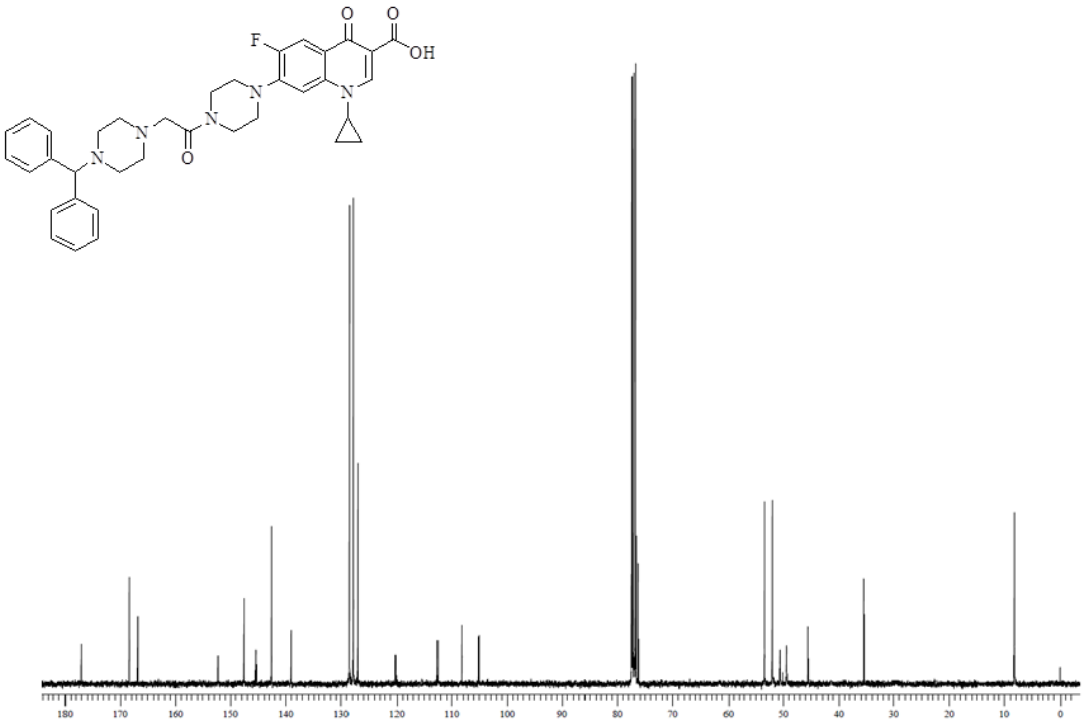
CP (1.0 g, 3 mmol) and triethylamine (0.4 mL, 3 mmol) were stirred in 10mL of dry dichloromethane (CH_2Cl_2) at 0 °C for 15 min under nitrogen (N_2) atmosphere. Chloroacetyl chloride (0.2mL, 3 mmol) was added drop wise slowly through a syringe. After stirring at 0 °C for 15 min, resultant mixture was warmed to room temperature (RT) and stirred for additional 1h. After the reaction was complete as indicated by TLC, 50ml of water was added and the compound was extracted from aqueous layer with 3×10 mL of CH_2Cl_2. The organic layers were collected, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resultant residue was purified by column chromatography (CH_2Cl_2/MeOH 2%) to yield the desired product (0.85 g, 70%) as a pale yellow solid. m.p. is 260-262 °C. ^{1}H-NMR (300 MHz,DMSO-d_6) δ 1.20 (t, *J* = 6.9 Hz, 2H), 1.32 (t, *J* = 7.2 Hz, 2H), 3.65 (m, 4H), 3.73 (m, 4H), 3.83 (tt, *J* = 7.2 Hz, *J* = 6.9 Hz, 1H), 4.46 (s, 2H), 7.59(d, $J_{H\text{-}F}$ = 7.52 Hz, 1H), 7.94 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 8.66 (s, 1H), 15.1 (s, 1H).

General procedure for the preparation of 1-cyclopropyl-6-fluoro-4-oxo-7-(4-substituted piperazin-1-yl)-1, 4-dihydro quinoline-3-carboxylic acid derivatives of CP ***(3a-v)***

To a solution of substituted piperazines (0.9819 mmol) in dry DMF (4mL), was added triethylamine (0.27mL, 1.9638 mmol) and potassium iodide (16.29mg, 0.0981 mmol) at RT under N_2 atmosphere. Then compound **2** (0.4g, 0.9819 mmol) was added and resultant mixture was heated for 5h at 125 °C. After the reaction was complete as indicated by TLC, DMF was evaporated under reduced pressure. The obtained residue was diluted with 20 mL of water. The compound was extracted from aqueous layer with 3×5 mL of CH_2Cl_2. The organic layers were collected, dried over anhydrous $MgSO_4$ and evaporated. The resultant residue was purified by column chromatography (CH_2Cl_2/MeOH (1-10%)).



^{1}H NMR spectrum of compound **3j**



^{13}C NMR spectrum of compound **3j**

1-cyclopropyl-6-fluoro-7-[4-(2-{4-methylpiperazin-1-yl}acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(3a)***

White solid; yield: 57%, 0.26g, m.p. 248-250 °C; IR (KBr, cm^{-1}) 3250, 3025, 1725, 1690, 1670, 1250, 1045. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 3.81 (tt, J = 7.2 Hz, J = 6.9 Hz, 1H), 3.78-3.76 (m, 4H), 3.30-3.28 (m, 2H), 3.19-3.16 (m, 4H), 3.02 (s, 3H), 2.92-2.89 (m, 4H), 2.69-2.67 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 61.21, 53.67, 51.12, 50.89, 49.16, 45.91, 43.78, 41.39, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{24}H_{30}FN_5O_4$ 471.23, found 472.39 $[M + H]^+$.

*7-[4-(2-{4-(4-chlorophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (****3b****)*

Pale yellow solid; yield: 68%, 0.37g, m.p. 249-250 °C; IR (KBr, cm^{-1}) 3265, 3018, 1719, 1686, 1675, 1245, 1050, 600-800. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.1 (d, J = 5.8 Hz, 2H), 6.52 (d, J = 8.5 Hz, 2H), 3.79 (tt, J = 7.2, 6.9 Hz, 1H), 3.69-3.67 (m, 4H), 3.35-3.32 (m, 2H), 3.29-3.26 (m, 4H), 3.01-2.98 (m, 4H), 2.69-2.67 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.84, 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 132.57, 125.51, 119.91 (d, J_{C-F} = 8.1 Hz), 116.38, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.89, 51.12, 50.46, 49.12, 45.84, 35.82, 8.43. ESI-MS (m/z): calcd. for $C_{29}H_{31}ClFN_5O_4$ 567.20, found 568.29 $[M + H]^+$.

*7-[4-(2-{4-acetylpiperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (****3c****)*

White solid; yield: 88%, 0.43g, m.p. 230-231 °C; IR (KBr, cm^{-1}) 3234, 3027, 1722, 1695, 1668, 1253, 1042. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 3.82 (tt, J = 7.2, 6.9 Hz, 1H), 3.79-3.76 (m, 4H), 3.34-3.31 (m, 2H), 3.23-3.21 (m, 4H), 2.88-2.86 (m, 4H), 2.54-2.52 (m, 4H), 2.15 (s, 3H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz),

168.39, 167.56, 153.21 (d, $J_{C\text{-}F}$ = 249.3 Hz), 147.23, 145.12 (d, $J_{C\text{-}F}$ = 10.3 Hz), 138.95, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 62.45, 52.83, 51.15, 48.86, 35.81, 25.14, 8.17. ESI-MS (m/z): calcd. for $C_{25}H_{30}FN_5O_5$ 499.22, found 500.42 $[M + H]^+$.

*7-[4-(2-{4-benzylpiperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3d**)*

White solid; yield: 89%, 0.47g, m.p. 104-106 °C; IR (KBr, cm^{-1}) 3267, 3036, 1730, 1696, 1675, 1251, 1067. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 7.52 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 7.32-7.30 (m, 5H), 3.71 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.65-3.62 (m, 4H), 3.48-3.46 (m, 2H), 3.34-3.32 (m, 4H), 3.15 (s, 2H), 2.96-2.93 (m, 4H), 2.74-2.72 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, $J_{C\text{-}F}$ = 2.2 Hz), 168.33, 153.6 (d, $J_{C\text{-}F}$ = 249.3 Hz), 146.21, 145.49 (d, $J_{C\text{-}F}$ = 10.3 Hz), 139.01, 127.12, 126.75, 126.13, 120.15 (d, $J_{C\text{-}F}$ = 8.1 Hz), 112.51 (d, $J_{C\text{-}F}$ = 24.14 Hz), 108.16, 105.08 (d, $J_{C\text{-}F}$ = 3.7 Hz), 61.41, 54.31, 51.17, 51.48, 49.11, 45.07, 35.12, 8.65. ESI-MS (m/z): calcd. for $C_{30}H_{34}FN_5O_4$ 547.26, found 548.62 $[M + H]^+$.

*1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-phenylpiperazin-1-yl}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3e**)*

White solid; yield: 88%, 0.46g, m.p. 212-214 °C; IR (KBr, cm^{-1}) 3247, 3031, 1732, 1694, 1672, 1252, 1038. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H). 8.63 (s, 1H), 7.89 (d, $J_{H\text{-}F}$ = 13.2 Hz, 1H), 7.52 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 7.26-7.23 (m, 5H), 3.81 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.71-3.68 (m, 4H), 3.46-3.44 (m, 2H), 3.30-3.28 (m, 4H), 2.93-2.91 (m, 4H), 2.64-2.62 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, $J_{C\text{-}F}$ = 2.2 Hz), 168.33, 166.82, 153.6 (d, $J_{C\text{-}F}$ = 249.3 Hz), 147.53, 145.49 (d, $J_{C\text{-}F}$ = 10.3 Hz), 144.21, 139.01, 130.09, 128.41, 127.74, 120.15 (d, $J_{C\text{-}F}$ = 8.1 Hz), 112.51 (d, $J_{C\text{-}F}$ = 24.14 Hz), 108.16, 105.08 (d, $J_{C\text{-}F}$ = 3.7 Hz), 54.65, 52.42, 51.17, 49.33, 45.40, 34.86, 8.18. ESI-MS (m/z): calcd. for $C_{29}H_{32}FN_5O_4$ 533.24, found 534.31 $[M + H]^+$.

*7-[4-(2-{4-(2-chlorophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3f**)*

White solid; yield: 95%, 0.53g, m.p. 138-139°C; IR (KBr, cm^{-1}) 3238, 3021, 1718, 1689, 1672, 1251, 1087, 728. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.58-7.55 (m, 2H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.13 (d, J = 7.7 Hz, 1H), 7.05 (t, J = 7.8 Hz, 1H), 3.76 (tt, J = 7.2 Hz, J = 6.9 Hz, 1H), 3.74-3.72 (m, 4H), 3.39-3.37 (m, 2H), 3.20-3.18 (m, 4H), 2.84-2.82 (m, 4H), 2.58-2.56 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 143.84, 139.01, 130.95, 129.13, 128.64, 124.2, 122.26, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F} = 3.7 Hz), 53.11, 51.75, 50.83, 48.96, 44.72, 35.42, 7.87. ESI-MS (m/z): calcd. for $C_{29}H_{31}ClFN_5O_4$ 567.20, found 568.35 $[M + H]^+$.

*7-[4-(2-{4-(4-cyanophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3g**)*

White solid; yield: 68%, 0.37g, m.p. 278-279 °C; IR (KBr, cm^{-1}) 3282, 3018, 2245, 1741, 1682, 1668, 1245, 1067. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.3 (d, J = 5.8 Hz, 2H), 6.65 (d, J = 8.5 Hz, 2H), 3.71 (tt, J = 7.2 Hz, J = 6.9 Hz, 1H), 3.70-3.67 (m, 4H), 3.34-3.32 (m, 2H), 3.23-3.21 (m, 4H), 2.81-2.79 (m, 4H), 2.48-2.45 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.84, 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 131.86, 126.93, 119.91 (d, J_{C-F} = 8.1 Hz), 117.12, 116.91, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.43, 51.75, 50.22, 49.72, 45.89, 35.23, 7.13. ESI-MS (m/z): calcd. for $C_{30}H_{31}FN_6O_4$ 558.24, found 559.38 $[M + H]^+$.

*1-cyclopropyl-6-fluoro-7-[4-(2-{4-(4-nitrophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3h**)*

Yellow solid; yield: 92%, 0.52g, m.p. 242-244 °C; IR (KBr, cm^{-1}) 3265, 3035, 1732, 1675, 1655, 1512, 1375, 1253, 1044. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.9 (d, J = 5.8 Hz, 2H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 6.7 (d, J = 8.5 Hz, 2H), 3.79 (tt, J = 7.2 Hz, J = 6.9 Hz, 1H), 3.71-3.69 (m, 4H), 3.41-3.38 (m, 2H), 3.25-3.23 (m, 4H), 2.85-2.83 (m, 4H), 2.56-2.54 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.82 (d,

J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 148.42, 146.93, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 132.45, 128.41, 119.91 (d, J_{C-F} = 8.1 Hz), 117.12, 116.91, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.39, 51.52, 50.79, 49.23, 35.85, 8.34. ESI-MS (*m/z*):calcd. for $C_{29}H_{31}FN_6O_6$ 578.23, found 579.32 $[M + H]^+$.

*1-cyclopropyl-6-fluoro-7-[4-(2-{4-(3-methoxyphenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3i**)*

Pale brown solid; yield: 87%, 0.48g, m.p. 144-145°C; IR (KBr, cm^{-1}) 3243, 3024, 1718, 1686, 1671, 1252, 1145, 1052. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.15 (t, *J* = 7.4 Hz, 1H), 6.57 (d, *J* = 7.3 Hz, 1H), 6.5 (s, 1H), 6.42 (d, *J* = 7.3 Hz, 1H), 3.79 (tt, 1H, *J* = 7.2 Hz, *J* = 6.9 Hz), 3.76 (s, 3H), 3.71-3.69 (m, 4H), 3.48-3.46 (m, 2H), 3.17-3.14 (m, 4H), 2.75-2.72 (m, 4H), 2.43-2.41 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 146.27, 145.49 (d, J_{C-F} = 10.3 Hz), 144.56, 139.01, 131.29, 129.72, 129.13, 123.18, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 110.3, 108.16, 105.08 (d, J_{C-F}= 3.7 Hz), 53.85, 52.11, 51.73, 48.17, 46.14, 44.72, 35.42, 8.18. ESI-MS (*m/z*):calcd. for $C_{30}H_{34}FN_5O_5$ 563.25, found 564.42 $[M + H]^+$.

*7-[4-(2-{4-benzhydrylpiperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3j**)*

White solid; yield: 82%, 0.50g, m.p. 199-200 °C, IR (KBr, cm^{-1}) 3265, 3018, 1723, 1695, 1668, 1246, 1038. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.27-7.25 (m, 10H), 4.12 (s, 1H), 3.83 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.72-3.69 (m, 4H), 3.48-3.46 (m, 2H), 3.21-3.18 (m, 4H), 2.96 (s, 3H), 2.70-2.68 (m, 4H), 2.45-2.43 (m, 4H), 1.32 (t, *J* = 6.9 Hz, 2H), 1.20 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 142.60, 139.01, 128.52, 127.86, 127.00, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F}= 3.7 Hz), 76.27, 53.35, 51.92, 50.65, 49.45, 45.48, 35.33, 8.26. ESI-MS (*m/z*):calcd. for $C_{36}H_{38}FN_5O_4$ 623.29, found 624.41 $[M + H]^+$.

*1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-m-tolylpiperazin-1-yl}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3k**)*

White solid; yield: 65%, 0.34g, m.p. 158-160°C; IR (KBr, cm^{-1}) 3268, 3054, 1719, 1697, 1668, 1251, 1082. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.18 (s, 1H), 8.73 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.42 (d, J_{H-F} = 7.5 Hz, 1H), 7.04 (t, *J* = 7.6 Hz, 1H), 6.71 (s, 1H), 6.68 (d, *J* = 7.5 Hz, 1H), 6.55 (d, *J* = 7.6 Hz, 1H), 3.76 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.74-3.72 (m, 4H), 3.43-3.41 (m, 2H), 3.40-3.38 (m, 4H), 2.68-2.66 (m, 4H), 2.40-2.38 (m, 4H), 2.19 (s, 3H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 146.03, 145.49 (d, J_{C-F} = 10.3 Hz), 141.48, 139.01, 129.5, 127.59, 127.11, 126.58, 122.34, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F}= 3.7 Hz), 53.87, 51.12, 50.86, 49.43, 45.12, 35.39, 22.29, 8.18. ESI-MS (*m/z*):calcd. for $C_{30}H_{34}FN_5O_4$ 547.26, found 548.39 $[M + H]^+$.

*1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(1-phenylethyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (3**l**)*

White solid; yield: 94%, 0.51g, m.p. 212-214 °C; IR (KBr, cm^{-1}) 3239, 3026, 1728, 1696, 1672, 1254, 1076. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.30-7.28 (m, 5H), 3.78 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.64-3.62 (m, 4H), 3.38-3.36 (m, 2H), 3.33-3.31 (m, 4H), 3.06 (q, *J* = 6.9 Hz, 1H), 2.54-2.52 (m, 4H), 2.41-2.39 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.20 (d, *J* = 5.9 Hz, 3H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 168.33, 166.82, 153.6 (d, J_{C-F} = 249.3 Hz), 147.53, 145.49 (d, J_{C-F} = 10.3 Hz), 142.60, 139.01, 128.52, 127.86, 127.00, 120.15 (d, J_{C-F} = 8.1 Hz), 112.51 (d, J_{C-F} = 24.14 Hz), 108.16, 105.08 (d, J_{C-F}= 3.7 Hz), 72.21, 54.11, 52.19, 51.07, 48.69, 46.18, 34.04, 22.67, 8.13. ESI-MS (*m/z*): calcd. for $C_{31}H_{36}FN_5O_4$ 561.28, found 562.41 $[M + H]^+$.

*1-cyclopropyl-7-[4-(2-{4-ethylpiperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3m**)*

White solid; yield: 94%, 0.44g, m.p. 239-240 °C; IR (KBr, cm^{-1}) 3287, 3032, 1734, 1698, 1672, 1253, 1048. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H),7.55 (d, J_{H-F} = 7.5 Hz, 1H), 3.79 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.69-3.67 (m, 4H), 3.54-3.52

(m, 2H), 3.35-3.32 (m, 4H), 2.9 (q, *J* = 6.1 Hz, 2H), 2.51-2.48 (m, 4H), 2.38-2.36 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H), 1.08 (t, *J* = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 54.56, 52.23, 51.67, 49.71, 48.87, 44.76, 34.74, 14.87, 8.45. ESI-MS (*m/z*): calcd. for $C_{25}H_{32}FN_5O_4$ 485.24, found 486.37 $[M + H]^+$.

*7-[4-(2-{4-(2-cyanophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3n**)*

White solid; yield: 63%, 0.34g, m.p. 222-223 °C; IR (KBr, cm^{-1}) 3245, 3022, 2238, 1729, 1692, 1671, 1255, 1043. ^{1}H NMR (300 MHz, DMSO- d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.92 (d, J_{H-F} = 13.2 Hz, 1H), 7.66 (d, J_{H-F} = 7.5 Hz, 1H), 7.57-7.55 (m, 2H), 7.13 (d, *J* = 6.9 Hz, 1H), 7.05 (t, *J* = 7.1 Hz, 1H), 3.82 (tt, 1H, *J* = 7.2, 6.9 Hz), 3.79-3.77 (m, 4H), 3.68-3.66 (m, 2H), 3.36-3.34 (m, 4H), 3.16-3.14 (m, 4H), 2.65-2.62 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 142.43, 138.95, 128.2, 127.43, 126.32, 122.78, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 108.23, 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 81.42, 53.59, 51.12, 50.58, 49.33, 45.06, 35.99, 8.54. ESI-MS (*m/z*): calcd. for $C_{30}H_{31}FN_6O_4$ 558.24, found 559.36 $[M + H]^+$.

*1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2- {4-(pyridine-2-yl)piperazin-1-yl}acetyl) piperazin-1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3o**)*

White solid; yield: 81%, 0.42g, m.p. 204-205 °C; IR (KBr, cm^{-1}) 3268, 3028, 1729, 1695, 1678, 1253, 1054. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.92 (d, J_{H-F} = 13.2 Hz, 1H), 7.66 (d, J_{H-F} = 7.5 Hz, 1H), 7.65-7.62 (m, 2H), 7.21 (d, *J* = 6.9 Hz, 1H), 7.16 (t, *J* = 6.8 Hz, 1H), 3.77 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.63-3.61 (m, 4H), 3.58-3.56 (m, 2H), 3.33-3.31 (m, 4H), 3.26-3.23 (m, 4H), 2.59-2.57 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 154.34, 153.21 (d, J_{C-F} = 249.3 Hz), 150.78, 148.43, 147.23, 146.07, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 121.56, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 53.78, 53.12,

50.96, 48.76, 45.78, 37.23, 8.45. ESI-MS (*m/z*): calcd. for $C_{28}H_{31}FN_6O_4$ 534.24, found 535.36 $[M + H]^+$.

*1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(pyrimidine-2-yl)piperazin-1-l}acetyl)piperazin- 1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3p**)*

Pale yellow solid; yield: 91%, 0.47g, m.p. 189-190 °C; IR (KBr, cm^{-1}) 3278, 3018, 1724, 1698, 1669, 1234, 1067. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 8.31 (d, J = 4.6 Hz, 2H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.55 (d, J_{H-F} = 7.5 Hz, 1H), 7.01(t, J = 6.8 Hz, 1H), 3.77 (tt, J = 7.2, 6.9 Hz, 1H), 3.69-3.67 (m, 4H), 3.36-3.34 (m, 2H), 3.07-3.05 (m, 4H), 2.60-2.58 (m, 4H), 2.48-2.46 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 160.67, 154.89, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 122.45, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 54.12, 52.23, 51.08, 49.67, 44.97, 36.24, 8.14. ESI-MS (*m/z*): calcd. for $C_{27}H_{30}FN_7O_4$ 535.23, found 536.39 $[M + H]^+$.

*1-cyclopropyl-7-[4-(2-{4-(3,4-dimethoxyphenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3q**)*

White solid; yield: 69%, 0.40g, m.p. 189-190 °C; IR (KBr, cm^{-1}) 3250, 3025, 1725, 1690, 1670, 1250, 1187, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.55 (d, J_{H-F} = 7.5 Hz, 1H), 6.63 (d, J = 6.8 Hz, 1H), 6.56 (d, J = 6.9 Hz, 1H), 6.25 (s, 1H), 3.79 (tt, J = 7.2, 6.9 Hz, 1H), 3.73 (s, 6H), 3.73-3.71 (m, 4H), 3.41-3.38 (m, 2H), 3.27-3.25 (m, 4H), 2.45-2.42 (m, 4H), 2.37-2.35 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 143.24, 140.67, 138.08, 138.95, 121.78, 116.02, 119.91 (d, J_{C-F} = 8.1 Hz), 117.32, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 61.45, 60.95, 59.34, 53.35, 51.92, 50.65, 49.45, 35.33, 8.26. ESI-MS (*m/z*): calcd. for $C_{31}H_{36}FN_5O_6$ 593.26, found 594.39 $[M + H]^+$.

*1-cyclopropyl-6-fluoro-7-[4-(2-{4-(3-hydroxyphenyl)piperazin-1-yl}acetylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3r**)*

White solid; yield: 79%, 0.42g, m.p. 220-222 °C; IR (KBr, cm^{-1}) 3450, 3245, 3026, 1732, 1692, 1669, 1236, 1067. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.18 (s, 1H), 8.63 (s, 1H), 7.89 (d, J_{H-F} = 13.2 Hz, 1H), 7.52 (d, J_{H-F} = 7.5 Hz, 1H), 7.05 (t, J = 6.9 Hz, 1H), 6.52 (d, J = 6.6 Hz, 1H), 6.45 (s, 1H), 6.39 (d, J = 6.7 Hz, 1H), 5.23 (s, 1H), 3.81 (tt, J = 7.2, 6.9 Hz, 1H), 3.67-3.65 (m, 4H), 3.48-3.46 (m, 2H), 3.36-3.34 (m, 4H), 2.45-2.43 (m, 4H), 2.35-2.32 (m, 4H), 1.29 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 146.58, 145.12 (d, J_{C-F} = 10.3 Hz), 142.03, 138.95, 134.61, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.28, 107.12, 106.07, 104.89 (d, J_{C-F} = 3.7 Hz), 101.72, 54.15, 50.91, 51.27, 49.81, 44.73, 35.99, 8.12. ESI-MS (*m/z*): calcd. for $C_{29}H_{32}FN_5O_5$ 549.24, found 550.45 $[M + H]^+$.

*1-cyclopropyl-7-[4-(2-{4-(3,4-difluorophenyl)piperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3s**)*

Pale yellow solid; yield: 72%, 0.40g, m.p. 198-200 °C; IR (KBr, cm^{-1}) 3256, 3023, 1721, 1696, 1675, 1252, 1044. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.22 (s, 1H), 8.71 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.63 (d, J_{H-F} = 7.5 Hz, 1H), 7.25 (dd, J_{H-F} = 12.11, 7.01 Hz, 1H), 7.15 (dt, J_{H-F} = 13.2, 6.8 Hz, 1H), 6.98 (dd, J_{H-F} = 7.1, 3.4 Hz, 1H), 3.85 (tt, J = 7.2, 6.9 Hz, 1H), 3.81-3.79 (m, 4H), 3.41-3.39 (m, 2H), 3.38-3.35 (m, 4H), 2.58-2.56 (m, 4H), 2.35-2.32 (m, 4H), 1.37 (t, J = 6.9 Hz, 2H), 1.24 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 162.58 (dd, J_{C-F} = 252.67 Hz, 22.68 Hz),153.21 (d, J_{C-F} = 249.3 Hz), 151.52 (dd, J_{C-F} = 247.28 Hz, 21.39 Hz),147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 141.49 (d, J_{C-F} = 2.8 Hz), 138.95, 125.23 (d, J_{C-F} = 3.07 Hz), 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 110.87 (dd, J_{C-F} = 23.81 Hz, 3.7 Hz), 109.42 (dd, J_{C-F} = 20.65 Hz, 2.95 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 55.32, 51.49, 51.11, 49.72, 45.29, 35.37, 8.42. ESI-MS (*m/z*): calcd. for $C_{29}H_{30}F_3N_5O_4$ 569.22, found 570.49 $[M + H]^+$.

*7-[4-(2-{4-(bis(4-fluoroophenyl)methyl)piperazin-1-yl}acetyl)piperazin-1-yl]-1-cyclo propyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3t**)*

White solid; yield: 66%, 0.42g, m.p. 195-196 °C; IR (KBr, cm^{-1}) 3265, 3045, 1724, 1686, 1672, 1251, 1044. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.22 (s, 1H), 8.71 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.61 (d, J_{H-F} = 7.5 Hz, 1H), 7.51 (dd, J_{H-F} = 5.8, 7.5 Hz, 4H), 7.21 (dd, J_{H-F} = 8.5 Hz,

4H), 4.2 (s, 1H), 3.86 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.76-3.74 (m, 4H), 3.35-3.32 (m, 2H), 3.27-3.25 (m, 4H), 2.56-2.54 (m, 4H), 2.39-2.37 (m, 4H), 1.32 (t, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 176.97 (d, J_{C-F} = 2.2 Hz), 168.23, 166.76, 161.83 (d, J_{C-F} = 246.49 Hz), 153.57 (d, J_{C-F} = 251.52 Hz), 147.50, 145.45 (d, = 10.3 Hz), 139.01, 138.07 (d, J_{C-F} = 2.9 Hz), 129.21 (d, J_{C-F} = 7.3 Hz), 120.08 (d, d, J_{C-F} = 8.1 Hz), 115.43 (d, J_{C-F}= 21.27 Hz), 112.46 (d, J_{C-F} = 23.48 Hz), 108.11, 105.05 (d, J_{C-F} = 3.7 Hz), 74.43, 53.31, 51.70, 50.61, 49.39, 45.45, 35.32, 8.25. ESI-MS (*m/z*): calcd. for $C_{36}H_{36}F_3N_5O_4$ 659.27, found 660.62 $[M + H]^+$.

*1-cyclopropyl-7-[4-(2-{4-(2-(dimethylamino)ethyl)piperazin-1-yl}acetyl)piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3u**)*

White solid, (0.38g, 75%), m.p. 138-140 °C; IR (KBr, cm^{-1}) 3248, 3025, 1728, 1696, 1672, 1250, 1045. ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.22 (s, 1H), 8.71 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.63 (d, J_{H-F} = 7.5 Hz, 1H), 3.89 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.78-3.76 (m, 4H), 3.41-3.39 (m, 2H), 3.38-3.35 (m, 4H), 2.83 (s, 6H), 2.70 (t, *J* = 7.2 Hz, 4H), 2.59-2.57 (m, 4H), 2.44-2.42 (m, 4H), 1.37 (t, *J* = 6.9 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.95, 53.53, 51.67, 50.22, 49.20, 48.63, 47.97, 41.11, 35.12, 8.09. ESI-MS (*m/z*): calcd. for $C_{27}H_{37}FN_6O_4$ 528.29, found 530.41 $[M + H]^+$.

*1-cyclopropyl-6-fluoro-4-oxo-7-[4-(2-{4-(3-(trifluoromethyl)phenyl)piperazin-1-yl}acetyl) piperazin -1-yl]-1,4-dihydroquinoline-3-carboxylic acid (**3v**)*

White solid; yield: 56%, 0.33g, m.p. 190-192 °C; IR (KBr, cm^{-1}) 3278, 3028, 1732, 1698, 1674, 1252, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.22 (s, 1H), 8.71 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.63 (d, J_{H-F} = 7.5 Hz, 1H), 7.48 (t, *J* = 8.03 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.26 (s, 1H), 7.14 (d, *J* = 7.78 Hz, 1H), 3.89 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.78-3.76 (m, 4H), 3.42-3.39 (m, 2H), 3.37-3.35 (m, 4H), 2.61-2.59 (m, 4H), 2.44-2.42 (m, 4H), 1.37 (t, *J* = 6.9 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 2H). ^{13}CNMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 148.38 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 132.94, 126.39, 124.62, 119.91 (d, J_{C-F} = 8.1 Hz), 119.19, 116.47, 112.72, 111.97 (d, J_{C-F} = 24.14 Hz),

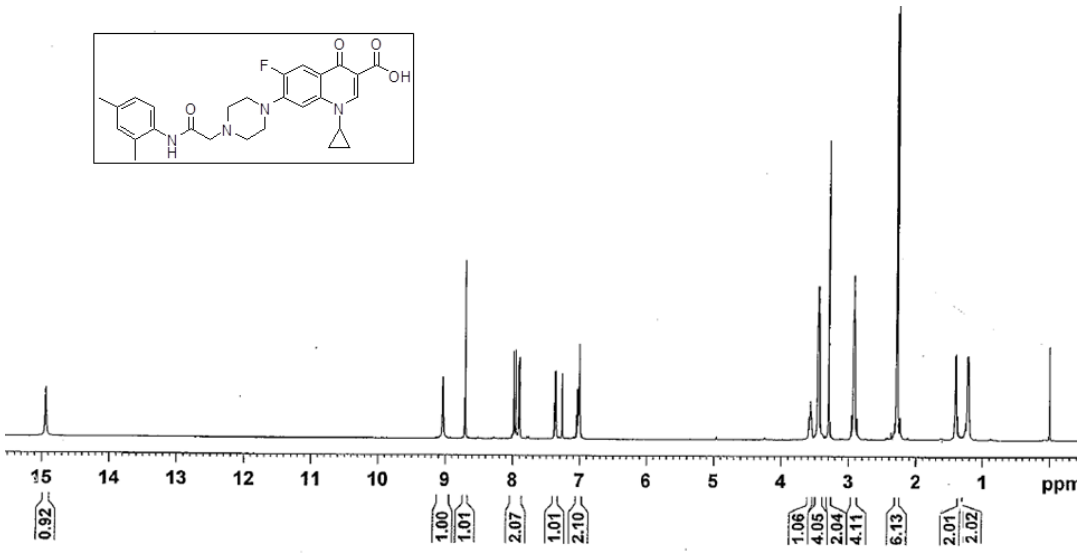
107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 54.11, 50.89, 50.16, 49.92, 45.14, 35.49, 8.08. ESI-MS (*m/z*): calcd. for $C_{30}H_{31}F_4N_5O_4$ 601.23, found 602.56 $[M + H]^+$

*Synthesis of 2-chloro-N-(substituted phenyl)acetamide (**5a-r**)*

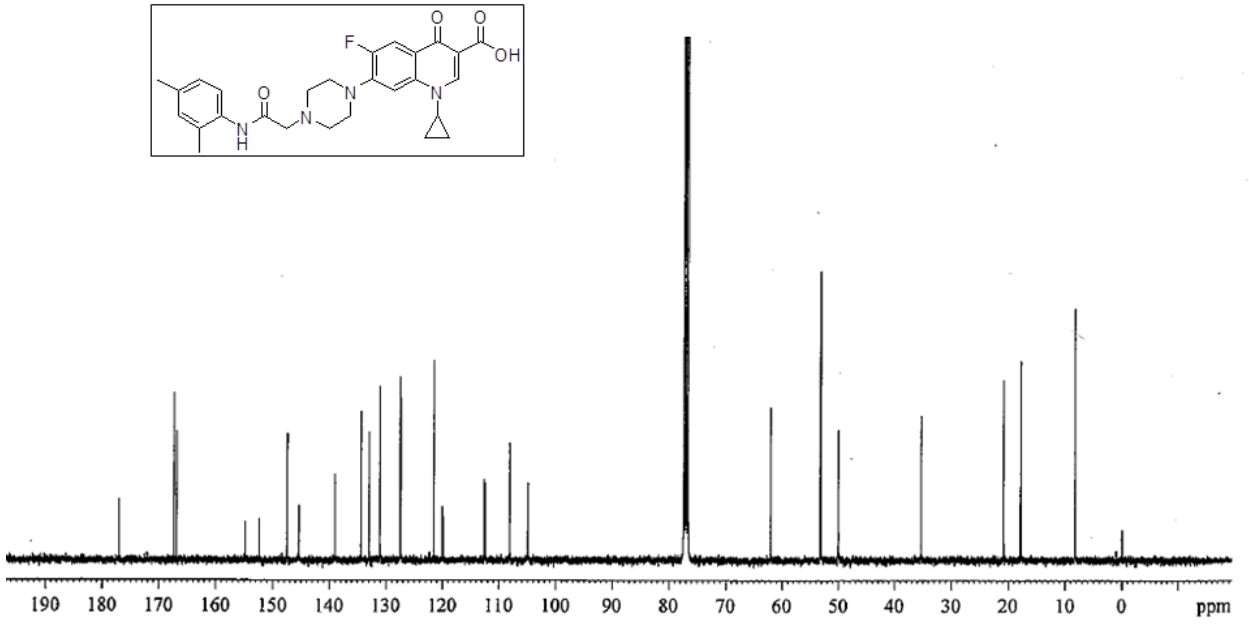
2-Chloroacetyl chloride (24 mmol) was slowly added dropwise to a mixture of various anilines (20 mmol) and Et_3N (24 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for an additional 20h. After the solvent was removed under reduced pressure, the residue was washed with ice water, and the precipitate was separated by filtration. The crude product was purified by crystallization using mixture of ether/hexane (**5a-r**).

*Synthesis of title compounds (**6a-r**)*

To a solution of **CP** (0.6036 mmol) in dry DMF (2 mL), triethylamine (1.8108 mmol) and potassium iodide (0.0603 mmol) were added at RT under N_2 atmosphere. To the resultant mixture, **5a** (0.6036 mmol) was added and heated at 125 °C. After the reaction was complete, as indicated by TLC. The obtained residue was diluted with 20 mL of water. The compound was extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resultant crude was purified by column chromatography [CH_2Cl_2/MeOH (1-10%)] to get the title compounds (**6a-r**).



^{1}H NMR spectrum of compound **6o**



^{13}C NMR spectrum of compound **6o**

7-(4-(2-(4-chlorophenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6a)***

White solid; yield: 73%, 0.32g, m.p. 222-224 °C; IR (KBr, cm^{-1}) 3375, 3254, 3025, 1725, 1690, 1670, 1250, 1045, 725. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.97 (s, 1H), 9.05 (s, 1H), 8.74 (s, 1H), 7.99 (d, $J_{H\text{-}F}$ = 12.7 Hz, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.39 (d, $J_{H\text{-}F}$ = 7.0 Hz, 1H), 7.29 (d, J = 8.7 Hz, 2H), 3.56 (tt, J = 7.0, 4.0 Hz, 1H), 3.46-3.44 (m, 4H), 3.27 (s, 2H), 2.91-2.89 (m, 4H), 1.28 (t, J = 6.7 Hz, 2H), 1.22 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, $J_{C\text{-}F}$ = 2.2 Hz), 167.56, 166.43, 153.21 (d, $J_{C\text{-}F}$ = 249.3 Hz), 147.23, 145.12 (d, $J_{C\text{-}F}$ = 10.3 Hz), 138.95, 136.61, 133.31, 129.62, 120.42, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}ClFN_4O_4$ 498.14, found 499.23 $[M + H]^+$.

7-(4-(2-(3-chlorophenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6b)***

White solid; yield: 67%, 0.30g, m.p. 249-251 °C; IR (KBr, cm^{-1}) 3375, 3250, 3025, 1725, 1690, 1670, 1250, 1045, 734. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.01 (s, 1H), 9.15 (s, 1H), 8.75 (s, 1H),

7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.85 (s, 1H), 7.45 (d, J = 8.6 Hz, 1H),7.40 (d, J_{H-F} = 7.5 Hz, 1H), 7.33 (t, J = 7.4, 1H), 7.25 (d, J = 8.6 Hz, 1H), 3.60 (tt, J = 7.2, 4.0 Hz, 1H), 3.43-3.41 (m, 4H), 3.28 (s, 2H), 2.91-2.89 (m, 4H), 1.32 (t, J = 6.7 Hz, 2H), 1.22 (t, 2H, J = 7.2 Hz). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 136.61, 134.21, 133.31, 129.62, 127.90, 122.91, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}ClFN_4O_4$ 498.14, found 499.28 $[M + H]^+$.

7-(4-(2-(2-chlorophenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6c)***

White solid; yield: 63%, 0.28g, m.p. 289-290 °C; IR (KBr, cm^{-1}) 3380, 3254, 3020, 1720, 1695, 1675, 1245, 1040, 745. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.01 (s, 1H), 9.15 (s, 1H), 8.75 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.75 (d, J = 8.6 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.43 (t, J = 6.9 Hz, 1H), 7.40 (d, J_{H-F} = 7.5 Hz, 1H), 7.35 (t, J = 7.2 Hz, 1H), 3.60 (tt, J = 7.2, 4.0 Hz, 1H), 3.42-3.40 (m, 4H), 3.28 (s, 2H), 2.99-2.91 (m, 4H), 1.32 (t, J = 6.7 Hz, 2H), 1.22 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 136.61, 134.21, 131.31, 126.42, 122.49, 121.91, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 112.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}ClFN_4O_4$ 498.14, found 499.32 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-(2-(3-methoxyphenylamino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6d)***

White solid; yield: 77%, 0.34g, m.p. 219-221 °C; IR (KBr, cm^{-1}) 3370, 3245, 3020, 1725, 1686, 1670, 1250, 1172, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.96 (s, 1H), 9.00 (s, 1H), 8.77 (s, 1H), 8.02 (d, J_{H-F} = 12.8 Hz, 1H), 7.40 (d, J = 7.5 Hz, 1H), 7.34 (t, J = 7.3 Hz, 1H), 7.24 (d, J_{H-F} = 8.3 Hz, 1H), 7.06 (d, J = 9.0 Hz, 1H), 6.71 (d, J = 8.3 Hz, 1H), 3.83 (s, 3H), 3.56 (tt, J = 7.2, 4.0 Hz, 1H), 3.45-3.43 (m, 4H), 3.26 (s, 2H), 2.90-2.88 (m, 4H), 1.42 (t, J = 6.7 Hz, 2H), 1.25 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 139.61, 134.21, 129.62, 127.90, 122.91,

120.42, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 116.7, 111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 60.67, 54.8, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{27}FN_4O_5$ 494.19, found 495.29 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-(2-(4-methoxyphenylamino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6e)***

White solid; yield: 67%, 0.29g, m.p. 208-210 °C; IR (KBr, cm^{-1}) 3376, 3252, 3024, 1722, 1690, 1675, 1235, 1165, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.97 (s, 1H), 8.89 (s, 1H), 8.74 (s, 1H), 8.02 (d, $J_{H\text{-}F}$= 12.8 Hz, 1H), 7.50 (d, *J* = 9.0 Hz, 2H), 7.39 (d, $J_{H\text{-}F}$ = 7.5 Hz, 1H), 6.89 (d, *J* = 8.3 Hz, 2H), 3.80 (s, 3H), 3.56 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.46-3.44 (m, 4H), 3.26 (s, 2H), 2.90-2.87 (m, 4H), 1.28 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.62 (d, $J_{C\text{-}F}$ = 2.2 Hz), 167.36, 166.63, 153.31 (d, $J_{C\text{-}F}$ = 249.3 Hz), 147.23, 145.12 (d, $J_{C\text{-}F}$ = 10.3 Hz), 139.65, 134.61, 132.34, 122.62, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 115.67, 111.57 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 60.67, 55.18, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{27}FN_4O_5$ 494.19, found 495.23 $[M + H]^+$.

7-(4-(2-(3-chloro-4-fluorophenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6f)***

Pale yellow solid; yield: 64%, 0.29g, m.p. 245-246 °C; IR (KBr, cm^{-1}) 3365, 3240, 3035, 1720, 1698, 1670, 1250, 1045, 755. ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.15 (s, 1H), 9.08 (s, 1H), 8.72 (s, 1H), 8.06 (s, 1H), 7.93 (d, $J_{H\text{-}F}$ = 12.7 Hz, 1H), 7.89 (s, 1H), 7.54 (d, *J* = 6.7 Hz, 1H), 7.19 (t, *J* = 6.9 Hz, 1H), 3.56 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.46-3.44 (m, 4H), 3.27 (s, 2H), 2.91-2.89 (m, 4H), 1.28 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, $J_{C\text{-}F}$ = 2.2 Hz), 167.56, 166.43, 153.21 (d, $J_{C\text{-}F}$ = 249.3 Hz), 147.23, 145.12 (d, $J_{C\text{-}F}$ = 10.3 Hz), 148.95, 134.61, 133.21, 124.32, 123.42, 120.09, 119.91 (d, $J_{C\text{-}F}$ = 8.1 Hz), 113.56, 111.97 (d, $J_{C\text{-}F}$ = 24.14 Hz), 107.12, 104.89 (d, $J_{C\text{-}F}$ = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{23}ClF_2N_4O_4$ 516.13, found 517.32 $[M + H]^+$.

7-(4-(2-(2-bromophenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6g)***

Pale brown solid; yield: 65%, 0.31g, m.p. 284-285 °C; IR (KBr, cm^{-1}) 3370, 3255, 3020, 1720, 1696, 1677, 1245, 1046, 650. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.97 (s, 1H), 9.07 (s, 1H), 8.74 (s, 1H), 8.02 (d, J_{H-F} = 12.7 Hz, 1H),7.86 (d, *J* = 8.9 Hz, 1H), 7.71 (d, *J* = 8.9 Hz, 1H), 7.40 (d, J_{H-F} = 7.0 Hz, 1H), 7.31 (t, *J* = 6.9 Hz, 1H), 7.10 (t, *J* = 6.8 Hz, 1H), 3.56 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.46-3.44 (m, 4H), 3.27 (s, 2H), 2.91-2.89 (m, 4H), 1.28 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.05, 134.61, 132.31, 131.65, 127.62, 125.42, 119.91 (d, J_{C-F} = 8.1 Hz), 117.23, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}BrFN_4O_4$ 542.09, found 543.23 $[M + H]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-(phenylamino)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid ***(6h)***

White solid; yield: 82%, 0.34g, m.p. 259-261 °C; IR (KBr, cm^{-1}) 3375, 3250, 3025, 1725, 1690, 1670, 1250, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.96 (s, 1H), 9.02 (s, 1H), 8.72 (s, 1H), 8.01 (d, J_{H-F} = 12.7 Hz, 1H), 7.59 (d, *J* = 7.6 Hz, 2H), 7.37 (d, J_{H-F} = 7.0 Hz, 1H),7.33 (t, *J* = 6.9 Hz, 2H), 7.13 (t, *J* = 6.8 Hz, 1H), 3.56 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.46-3.44 (m, 4H), 3.27 (s, 2H), 2.91-2.89 (m, 4H), 1.38 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.50, 134.51, 129.02, 128.42, 122.45, 119.91 (d, J_{C-F} = 8.1 Hz), 117.83, 111.97 (d, J_{C-F} = 24.14 Hz), 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{25}FN_4O_4$ 464.18, found 464.29 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-(2-(methyl(phenyl)amino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6i)***

White solid; yield: 85%, 0.36g, m.p. 189-190 °C; IR (KBr, cm^{-1}) 3370, 3255, 3045, 1725, 1695, 1673, 1254, 1055. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.96 (s, 1H), 8.72 (s, 1H), 8.01 (d, J_{H-F} = 12.7 Hz, 1H), 7.53 (t, *J* = 6.8 Hz, 2H), 7.43 (t, *J* = 7.1 Hz, 1H), 7.39 (d, J_{H-F} = 7.0 Hz, 1H), 7.27 (d, *J* = 7.6 Hz, 2H), 3.56 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.49 (s, 3H), 3.46-3.44 (m, 4H), 3.27 (s, 2H), 2.91-2.88 (m, 4H), 1.28 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} =

10.3 Hz), 138.50, 134.51, 129.82, 127.42, 122.45, 119.91 (d, J_{C-F} = 8.1 Hz), 117.83, 111.97 (d, J_{C-F} = 24.14 Hz), 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 34.67, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{27}FN_4O_4$ 478.20, found 479.36 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-(2-(3-nitrophenylamino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6j)***

Yellow solid; yield: 82%, 0.37g, m.p. 265-266 °C; IR (KBr, cm^{-1}) 3382, 3254, 3025, 1730, 1690, 1675, 1525, 1370, 1250, 1035. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.01 (s, 1H), 9.15 (s, 1H), 8.75 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.85 (s, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.40 (d, J_{H-F} = 7.5 Hz, 1H), 7.33 (t, *J* = 7.1 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 1H), 3.60 (tt, *J* = 7.2, 4.0 Hz, 1H), 3.42-3.40 (m, 4H), 3.28 (s, 2H), 2.91-2.89 (m, 4H), 1.32 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 137.61, 135.21, 133.31, 128.62, 126.60, 122.91, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}FN_5O_6$ 509.18, found 510.32 $[M + H]^+$.

1-cyclopropyl-7-(4-(2-(ethyl(phenyl)amino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6k)***

White solid; yield: 64%, 0.28g, m.p. 180-182 °C; IR (KBr, cm^{-1}) 3375, 3250, 3025, 1725, 1690, 1670, 1250, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.96 (s, 1H), 8.72 (s, 1H), 8.01 (d, J_{H-F} = 12.7 Hz, 1H), 7.53 (t, *J* = 6.8 Hz, 2H), 7.43 (t, *J* = 6.9 Hz, 1H), 7.39 (d, J_{H-F} = 7.0 Hz, 1H), 7.27 (d, *J* = 7.6 Hz, 2H), 3.86 (q, *J* = 7.6 Hz, 2H), 3.56 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.47-3.45 (m, 4H), 3.27 (s, 2H), 2.92-2.90 (m, 4H), 1.35 (t, *J* = 7.6 Hz, 3H), 1.28 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.50, 134.51, 129.82, 127.42, 122.45, 119.91 (d, J_{C-F} = 8.1 Hz), 117.83, 111.97 (d, J_{C-F} = 24.14 Hz), 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 34.67, 13.56, 8.12. ESI-MS (m/z): calcd. for $C_{27}H_{29}FN_4O_4$ 492.20, found 493.36 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-(2-(4-nitrophenylamino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6l)***

Yellow solid; yield: 60%, 0.27g, m.p. 257-258 °C; IR (KBr, cm^{-1}) 3370, 3255, 3020, 1720, 1695, 1675, 1525, 1375, 1250, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.97 (s, 1H), 9.05 (s, 1H), 8.74 (s, 1H), 8.02 (d, J_{H-F} = 12.7 Hz, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.39 (d, J_{H-F} = 7.0 Hz, 1H), 7.31 (d, J = 8.7 Hz, 2H), 3.56 (tt, J = 7.0, 4.0 Hz, 1H), 3.47-3.44 (m, 4H), 3.27 (s, 2H), 2.91-2.89 (m, 4H), 1.28 (t, J = 6.7 Hz, 2H), 1.22 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 136.61, 133.31, 129.62, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}FN_5O_6$ 501.16, found 502.28 $[M + H]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-(3-(trifluoromethyl) phenylamino) ethyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid ***(6m)***

White solid; yield: 65%, 0.31g, m.p. 222-223 °C; IR (KBr, cm^{-1}) 3370, 3255, 3020, 1720, 1695, 1675, 1255, 1040. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.01 (s, 1H), 9.15 (s, 1H), 8.75 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.85 (s, 1H), 7.55 (d, J= 8.6 Hz, 1H), 7.40 (d, J_{H-F} = 7.5 Hz, 1H), 7.33 (t, J = 7.3 Hz, 1H), 7.15 (d, J = 8.6 Hz, 1H), 3.60 (tt, J = 7.2, 4.0 Hz, 1H), 3.42-3.40 (m, 4H), 3.28 (s, 2H), 2.92-2.90 (m, 4H), 1.32 (t, J = 6.7 Hz, 2H), 1.22 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 137.61, 135.21, 133.31, 128.62, 126.60, 125.34, 122.91, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F}= 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{24}F_4N_4O_4$ 532.18, found 533.32 $[M + H]^+$.

7-(4-(2-(3-chloro-2-methylphenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6n)***

Pale yellow solid; yield: 63%, 0.29g, m.p. 268-269 °C; IR (KBr, cm^{-1}) 3370, 3245, 3020, 1720, 1695, 1675, 1250, 1045, 780. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.01 (s, 1H), 9.15 (s, 1H), 8.75 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.45 (d, J = 8.6 Hz, 1H), 7.40 (d, J_{H-F} = 7.5 Hz, 1H), 7.23 (t, J = 7.4 Hz, 1H), 7.16 (d, J = 8.6 Hz, 1H), 3.60 (tt, J = 7.2, 4.0 Hz, 1H), 3.42-3.40 (m, 4H), 3.28 (s,

2H), 2.91-2.89 (m, 4H), 2.21 (s, 3H), 1.32 (t, J = 6.7 Hz, 2H), 1.22 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 137.61, 135.21, 134.31, 132.24, 127.62, 124.60, 119.91 (d, J_{C-F} = 8.1 Hz), 115.67, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 13.56, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{26}ClFN_4O_4$ 512.16, found 512.28 $[M + H]^+$.

1-cyclopropyl-7-(4-(2-(2,4-dimethylphenylamino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6o)***

White solid; yield: 65%, 0.28g, m.p. 256-258 °C, IR (KBr, cm^{-1}) 3370, 3257, 3020, 1720, 1695, 1675, 1250, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.95 (s, 1H), 9.03 (s, 1H), 8.70 (s, 1H), 7.98 (d, J = 8.6 Hz, 1H), 7.91 (d, J_{H-F} = 12.7 Hz, 1H), 7.37 (d, J_{H-F} = 7.5 Hz, 1H), 7.04 (d, J = 8.6 Hz, 1H), 7.00 (s, 1H), 3.56 (tt, J = 7.0, 4.0 Hz, 1H), 3.47-3.44 (m, 4H), 3.29 (s, 2H), 2.93-2.90 (m, 4H), 2.29 (s, 6H), 1.39 (t, J = 6.7 Hz, 2H), 1.22 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.99 (d, J_{C-F} = 2.2 Hz), 167.37, 166.87, 154.87 (d, J_{C-F} = 249.3 Hz), 152.35, 147.44, 145.47 (d, J_{C-F} = 10.3 Hz), 139.03, 134.40, 132.95, 131.08, 127.51, 121.50, 120.01 (d, J_{C-F} = 8.1 Hz), 112.61 (d, J_{C-F} = 24.14 Hz), 108.10, 104.93 (d, J_{C-F} = 3.7 Hz), 62.07, 53.22, 50.04, 35.31, 20.85, 17.83, 8.24. ESI-MS (m/z): calcd. for $C_{27}H_{29}FN_4O_4$ 492.21, found 493.32 $[M + H]^+$.

1-cyclopropyl-7-(4-(2-(2,5-dimethylphenylamino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6p)***

White solid; yield: 62%, 0.27g, m.p. 244-245 °C, IR (KBr, cm^{-1}) 3370, 3255, 3026, 1745, 1690, 1675, 1255, 1048. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.15 (s, 1H), 9.8 (s, 1H), 8.72 (s, 1H), 8.06 (s, 1H), 7.93 (d, J_{H-F} = 12.7 Hz, 1H), 7.29 (s,1H), 7.07 (d, J = 8.6 Hz, 1H), 6.83 (d, J = 8.6 Hz, 1H), 3.56 (tt, J = 7.0, 4.0 Hz, 1H), 3.46-3.43 (m, 4H), 3.27 (s, 2H), 2.91-2.89 (m, 4H), 2.34 (s, 3H), 2.12 (s, 3H), 1.28 (t, J = 6.7 Hz, 2H), 1.22 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 154.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 139.03, 136.87, 135.36, 130.19, 125.56, 123.32, 121.42, 119.94 (d, J_{C-F} = 8.1 Hz), 112.62 (d, J_{C-F} = 24.14 Hz), 108.12, 104.93 (d, J_{C-F} = 3.7 Hz), 62.13, 53.21, 50.05, 35.32, 21.24, 17.47, 8.24. ESI-MS (m/z): calcd. for $C_{27}H_{29}FN_4O_4$ 492.21, found 493.34 $[M + H]^+$.

1-cyclopropyl-7-(4-(2-(2,6-diethylphenylamino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6q)***

White solid; yield: 67%, 0.31g, m.p. 261-262 °C, IR (KBr, cm^{-1}) 3386, 3258, 3020, 1726, 1692, 1674, 1254, 1050. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.15 (s, 1H), 9.8 (s, 1H), 8.72 (s, 1H), 8.06 (s, 1H), 7.93 (d, J_{H-F} = 12.7 Hz, 1H), 7.37 (t, *J* = 7.6 Hz, 1H), 7.11 (d, *J* = 8.6 Hz, 2H), 3.56 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.47-3.44 (m, 4H), 3.27 (s, 2H), 2.91-2.89 (m, 4H), 2.60 (q, *J* = 7.5 Hz, 4H), 1.35 (t, *J* = 7.4 Hz, 6H), 1.28 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.98 (d, J_{C-F} = 2.2 Hz), 168.69, 166.90, 154.89 (d, J_{C-F} = 249.3 Hz), 147.44, 145.57 (d, J_{C-F} = 10.3 Hz), 141.08, 139.01, 132.24, 128.06, 126.52, 120.01 (d, J_{C-F} = 8.1 Hz), 112.31 (d, J_{C-F} = 24.14 Hz), 108.03, 104.96 (d, J_{C-F} = 3.7 Hz), 61.69, 53.55, 49.95, 35.33, 25.11, 14.57, 8.24. ESI-MS (m/z): calcd. for $C_{29}H_{33}FN_4O_4$ 520.24, found 521.34 $[M + H]^+$.

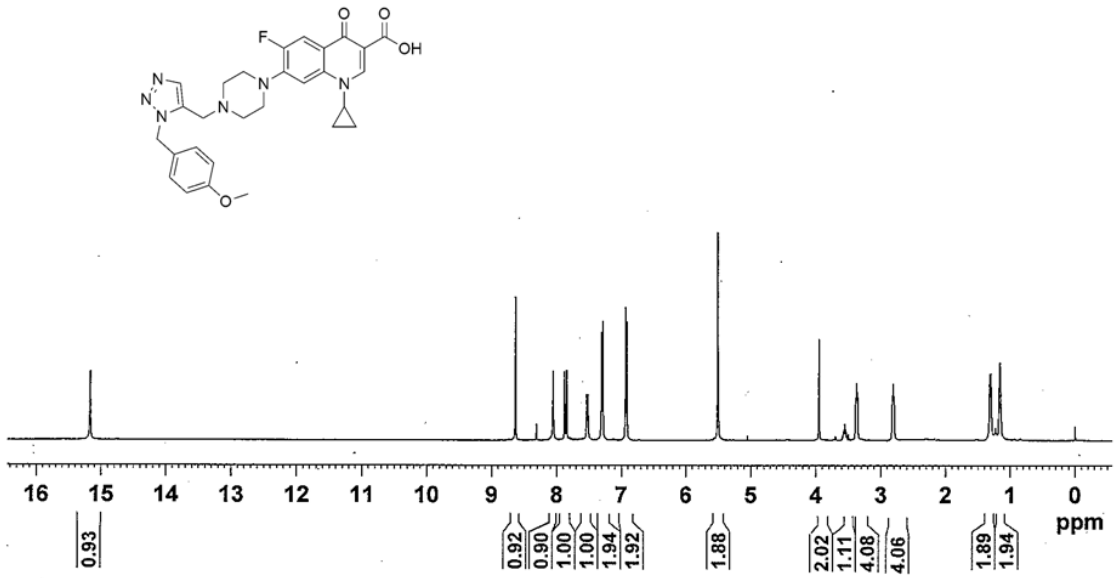
1-cyclopropyl-7-(4-(2-(3,4-dichlorophenylamino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(6r)***

Pale yellow solid; yield: 71%, 0.34g, m.p. 214-216 °C; IR (KBr, cm^{-1}) 3370, 3255, 3020, 1728, 1694, 1665, 1255, 1040, 740. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.15 (s, 1H), 9.8 (s, 1H), 8.72 (s, 1H), 8.06 (s, 1H), 7.93 (d, J_{H-F} = 12.7 Hz, 1H), 7.90 (s, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 1H), 3.56 (tt, *J* = 7.0, 4.0 Hz, 1H), 3.46-3.44 (m, 4H), 3.27 (s, 2H), 2.92-2.89 (m, 4H), 1.28 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.05, 134.61, 131.21, 130.32, 129.42, 124.09, 121.23, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{23}Cl_2FN_4O_4$ 532.10, found 533.22 $[M + H]^+$.

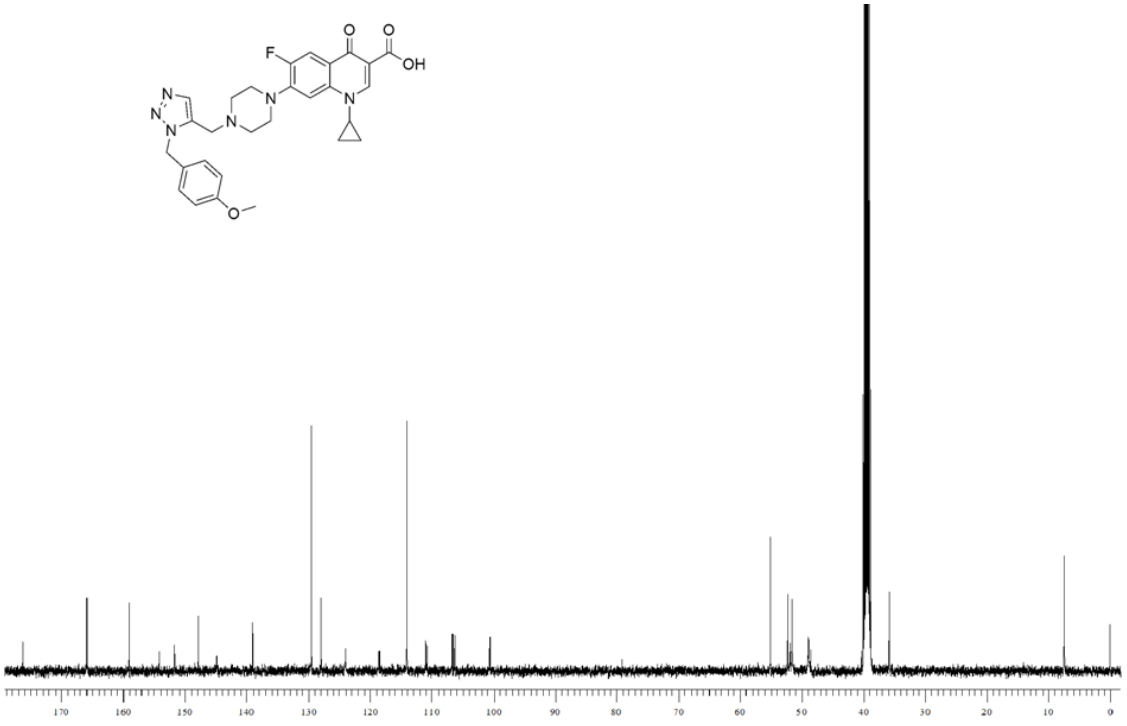
General procedure for ***(8a-x)***

To a stirred solution of compound **7** (0.4g, 1.0 mmol) and substituted phenyl azide (1.2 mmol) in tertiary butanol-water (1:1) (4 mL), $CuSO_4.5H_2O$ (1 mol %) (0.2 mmol) and sodium ascorbate (5 mol %) (0.2 mmol) were added and the reaction mixture was stirred at RT for 12 h. After completion of the reaction, as indicated by TLC, butanol was removed under reduced pressure. The residue was extracted with chloroform (3 x10 mL) and combined organic layers were collected and washed with saturated brine solution, dried over anhydrous $MgSO_4$ and

concentrated in vacuo to get the crude product. The product was further purified by column chromatography using dichloromethane and methanol (10%) to afford the title compounds.



^{1}H NMR spectrum of Compound **8c**



^{13}C NMR spectrum of Compound **8c**

7-(4-((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(8a)**

White solid; yield: 68%, 0.38g, m.p. 210-212 °C; IR (KBr, cm^{-1}) 3370, 3256, 3020, 1734, 1698, 1675, 1255, 1040, 775. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.13 (s, 1H), 8.77 (s, 1H), 8.12 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.62 (d, *J* = 5.8 Hz, 1H), 7.51-7.48 (m, 2H), 7.36 (d, *J* = 5.8 Hz, 1H), 3.97 (s, 2H), 3.59 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.46-3.44 (m, 4H), 2.84-2.82 (m, 4H), 1.38 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 151.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.85, 135.67, 134.81, 133.86, 132.76, 131.69, 130.75, 129.43, 127.91, 118.95 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.73, 52.49, 51.77, 49.16, 34.72, 8.18. ESI-MS (m/z): calcd. for $C_{26}H_{24}ClFN_6O_3$ 522.16, found 523.22 $[M + H]^+$.

7-(4-((1-(2-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8b**)

Pale yellow solid; yield: 79%, 0.48g, m.p. 228-229 °C; IR (KBr, cm^{-1}) 3370, 3255, 3020, 1725, 1690, 1670, 1250, 1040, 565. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.11 (s, 1H), 8.75 (s, 1H), 8.13 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.69 (d, J_{H-F} = 7.5 Hz, 1H), 7.60 (d, *J* = 5.8 Hz, 1H), 7.52-7.48 (m, 2H), 7.39 (d, *J* = 5.8 Hz, 1H), 3.92 (s, 2H), 3.56 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.42-3.40 (m, 4H), 2.83-2.81 (m, 4H), 1.39 (t, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 166.23, 152.21 (d, J_{C-F} = 249.3 Hz), 146.23, 145.12 (d, J_{C-F} = 10.3 Hz), 136.95, 135.57, 134.51, 133.36, 132.56, 131.19, 130.35, 129.45, 127.94, 118.91 (d, J_{C-F} = 8.1 Hz), 112.97 (d, J_{C-F} = 24.14 Hz), 109.89 (d, J_{C-F} = 3.7 Hz), 102.43, 52.89, 51.67, 49.12, 34.82, 8.13. ESI-MS (m/z): calcd. for $C_{26}H_{24}BrFN_6O_3$ 566.11, found 567.19 $[M + H]^+$.

1-cyclopropyl- 6-fluoro- 7-(4-((1-(4- methoxy benzyl) -1H-1,2,3-triazol-4-yl) methyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(8c)**

White solid; yield: 71%, 0.40g, m.p. 225-227 °C; IR (KBr, cm^{-1}) 3343, 3265, 3015, 1730, 1695, 1677, 1250, 1145, 1040. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.13 (s, 1H), 8.77 (s, 1H), 8.12 (s, 1H), 7.99 (d, J_{H-F} = 13.2 Hz, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.26 (d, *J* = 5.8 Hz, 2H), 6.82 (d, *J* = 8.5 Hz, 2H), 5.58 (s, 2H), 3.77 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.76-3.74 (m, 4H), 3.64 (s, 3H), 2.94-2.92 (m, 4H), 2.89 (s, 2H), 1.32 (t, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz,

$CDCl_3$) δ 176.23 (d, J_{C-F} = 2.2 Hz), 165.88, 159.05 (d, J_{C-F} = 249.3 Hz), 154.12, 151.64, 147.87, 144.92 (d, J_{C-F} = 10.3 Hz), 139.06, 129.52, 127.91, 124.01, 118.58 (d, J_{C-F} = 8.1 Hz), 114.05, 110.98 (d, J_{C-F} = 3.7 Hz), 106.32 (d, J_{C-F} = 24.14 Hz), 102.73, 55.07, 52.27, 51.87, 51.54, 48.9, 35.77, 7.58. ESI-MS (m/z): calcd. for $C_{28}H_{29}FN_6O_4$ 532.22, found 533.32 $[M + H]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-((1-phenethyl-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **(8d)**

White solid; yield: 73%, 0.40g, m.p. 190-191 °C; IR (KBr, cm^{-1}) 3363, 3250, 3035, 1735, 1699, 1670, 1241, 1052. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.12 (s, 1H), 8.74 (s, 1H), 8.15 (s, 1H), 7.96 (d, J_{H-F} = 13.2 Hz, 1H), 7.68 (d, J_{H-F} = 7.5 Hz, 1H), 7.49 (t, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 5.8 Hz, 2H), 7.29 (t, *J* = 8.5 Hz, 1H), 4.08 (t, *J* = 7.2 Hz, 2H), 3.89 (s, 2H), 3.54 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.38-3.36 (m, 4H), 3.14 (t, *J* = 6.9 Hz, 2H) 2.93-2.90 (m, 4H), 1.30 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.87 (d, J_{C-F} = 2.2 Hz), 166.33, 151.21 (d, J_{C-F} = 249.3 Hz), 147.23, 146.8, 145.82 (d, J_{C-F} = 10.3 Hz), 136.67, 135.81, 133.69, 129.63, 127.21, 124.67, 118.95 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.73, 57.42, 53.45, 51.77, 49.16, 35.24, 34.72, 8.18. ESI-MS (m/z): calcd. for $C_{28}H_{29}FN_6O_3$ 516.23, found 517.32 $[M + H]^+$.

7-(4-((1-(3-chloro phenyl)-1H-1,2,3-triazol-4-yl)methyl) piperazin-1-yl)-1-cyclo propyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8e**)

White solid; yield: 62%, 0.35g, m.p. 100-101 °C; IR (KBr, cm^{-1}) 3382, 3250, 3032, 1736, 1692, 1670, 1250, 1035, 745. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.12 (s, 1H), 8.73 (s, 1H), 8.18 (s, 1H), 7.95 (d, J_{H-F} = 13.2 Hz, 1H), 7.82 (s, 1H), 7.64 (d, J_{H-F} = 7.5 Hz, 1H), 7.51(d, *J* = 5.8 Hz, 2H), 7.4 (t, *J* = 5.8 Hz, 1H), 3.92 (s, 2H), 3.56 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.51-3.49 (m, 4H), 2.89-2.86 (m, 4H), 1.38 (t, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 151.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.45, 135.97, 134.81, 134.12, 133.96, 131.96, 128.43, 125.91, 124.65, 118.95 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.73, 52.49, 51.77, 49.16, 34.72, 8.18. ESI-MS (m/z): calcd. for $C_{26}H_{24}ClFN_6O_3$ 522.16, found 523.25 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl) methyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8f**)

White solid; yield: 61%, 0.34g, m.p. 154-155 °C; IR (KBr, cm^{-1}) 3375, 3250, 3025, 1730, 1695, 1675, 1250, 1162, 1055. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.11 (s, 1H), 8.78 (s, 1H), 8.18 (s, 1H), 7.95 (d, J_{H-F} = 13.2 Hz, 1H), 7.64 (d, J_{H-F} = 7.5 Hz, 1H), 7.44 (t, *J* = 5.8 Hz, 1H), 7.21(d, *J* = 5.8 Hz, 1H), 6.92 (d, *J* = 5.8 Hz, 1H), 6.82 (s, 1H), 3.95 (s, 2H), 3.82 (s, 3H), 3.54 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.45-3.42 (m, 4H), 2.92-2.89 (m, 4H), 1.33 (t, *J* = 6.9 Hz, 2H), 1.2 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 162.12, 151.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.82 (d, J_{C-F} = 10.3 Hz), 142.45, 135.97, 132.81, 130.12, 129.96, 128.43, 118.95 (d, J_{C-F} = 8.1 Hz), 115.91, 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 108.65, 102.73, 56.23, 52.49, 51.77, 49.16, 34.72, 8.18. ESI-MS (m/z): calcd. for $C_{27}H_{27}FN_6O_4$ 518.21, found 519.31 $[M + H]^+$.

7-(4-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8g**)

White solid; yield: 66%, 0.37g, m.p. 192-193 °C; IR (KBr, cm^{-1}) 3370, 3256, 3020, 1734, 1698, 1675, 1255, 1040, 763. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.1 (s, 1H), 8.81(s, 1H), 8.16 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.62 (d, *J* = 5.8 Hz, 2H), 7.46 (d, *J* = 5.8 Hz, 2H), 3.97 (s, 2H), 3.59 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.47-3.44 (m, 4H), 2.84-282 (m, 4H), 1.36 (t, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 151.21 (d, J_{C-F} = 249.3 Hz), 146.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.85, 134.81, 133.86, 132.76, 131.69, 129.43, 128.11, 118.95 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.73, 52.49, 51.77, 49.16, 34.72, 8.18. ESI-MS (m/z): calcd. for $C_{26}H_{24}ClFN_6O_3$ 522.16, found 523.25 $[M + H]^+$.

7-(4-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8h**)

Pale yellow solid; yield: 68%, 0.39g, m.p. 118-120 °C; IR (KBr, cm^{-1}) 3356, 3265, 3025, 1730, 1695, 1670, 1250, 1045, 745. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.19 (s, 1H), 8.69 (s, 1H), 8.47 (s, 1H),7.94 (d, J_{H-F} = 13.2 Hz, 1H), 7.73 (s, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.52 (d, *J* = 5.8 Hz, 1H), 7.16 (d, *J* = 5.8 Hz, 1H), 3.87 (s, 2H), 3.69 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.31-3.29 (m, 4H),

2.74-2.72 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.28 (d, J_{C-F} = 2.2 Hz), 165.92, 157.95, 151.21 (d, J_{C-F} = 249.3 Hz), 147.9, 145.11 (d, J_{C-F} = 10.3 Hz), 144.32, 139.1, 137.96, 133.68, 123.36, 122.42, 122.16, 120.61, 118.19 (d, J_{C-F} = 8.1 Hz), 111.91 (d, J_{C-F} = 24.14 Hz), 106.67 (d, J_{C-F} = 3.7 Hz), 102.76, 51.75, 49.31, 48.55, 35.77, 7.85. ESI-MS (m/z): calcd. for $C_{26}H_{23}ClF_2N_6O_3$ 540.15, found 541.23 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-((1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8i**)

Yellow solid; yield: 90%, 0.51g, m.p. 110-112 °C; IR (KBr, cm^{-1}) 3373, 3259, 3018, 1735, 1690, 1675, 1525, 1365, 1250, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.17 (s, 1H), 8.96 (s, 1H), 8.81 (s, 1H), 8.27(d, *J* = 5.8 Hz, 1H), 8.11 (s, 1H), 7.98 (d, *J* = 5.8 Hz, 1H), 7.78 (d, J_{H-F} = 13.2 Hz, 1H), 7.64 (d, J_{H-F} = 7.5 Hz, 1H), 7.51 (t, *J* = 5.8 Hz, 1H), 3.82 (s, 2H), 3.67 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.36-3.34 (m, 4H), 2.79-2.76 (m, 4H), 1.29 (t, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 151.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.82 (d, J_{C-F} = 10.3 Hz), 137.45, 136.97, 135.21, 134.12, 132.96, 131.96, 128.43, 125.91, 122.65, 118.95 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.73, 52.49, 51.77, 49.16, 34.75, 8.14. ESI-MS (m/z): calcd. for $C_{26}H_{24}FN_7O_5$ 533.18, found 534.27 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8j**)

White solid; yield: 51%, 0.27g, m.p. 144-145 °C; IR (KBr, cm^{-1}) 3375, 3250, 3025, 1730, 1695, 1670, 1255, 1055. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.1 (s, 1H), 8.81(s, 1H), 8.16 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.64 (d, *J* = 5.8 Hz, 2H), 7.29 (d, *J* = 5.8 Hz, 2H), 3.97 (s, 2H), 3.59 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.47-3.44 (m, 4H), 2.84-2.81 (m, 4H), 1.36 (t, *J* = 6.9 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 151.21 (d, J_{C-F} = 249.3 Hz), 146.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.65, 135.91, 133.86, 132.76, 130.89, 129.43, 127.81, 118.95 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.93, 52.49, 51.87, 49.26, 34.79, 8.21. ESI-MS (m/z): calcd. for $C_{26}H_{24}F_2N_6O_3$ 506.19, found 507.25 $[M + H]^+$.

1-cyclopropyl-7-(4-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8k**)

White solid; yield: 72%, 0.40g, m.p. 165-166 °C; IR (KBr, cm^{-1}) 3362, 3254, 3025, 1730, 1695, 1670, 1255, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.1 (s, 1H), 8.81(s, 1H), 8.16 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.54 (d, J = 5.8 Hz, 2H), 7.31 (d, J = 5.8 Hz, 2H), 3.97 (s, 2H), 3.59 (tt, J = 7.2, 6.9 Hz, 1H), 3.44-3.41 (m, 4H), 2.85-2.82 (m, 4H), 2.78-2.76 (m, 2H), 1.36 (t, J = 6.9 Hz, 2H), 1.25 (t, J = 7.2 Hz, 3H), 1.19 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 151.21 (d, J_{C-F} = 249.3 Hz), 146.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.65, 135.91, 133.86, 132.76, 130.93, 129.23, 127.82, 118.25 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.93, 52.42, 51.84, 49.26, 34.71, 29.34, 15.67, 8.21. ESI-MS (m/z): calcd. for $C_{28}H_{29}FN_6O_3$ 516.23, found 517.31 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8l**)

White solid; yield: 50%, 0.28g, m.p. 132-133 °C; IR (KBr, cm^{-1}) 3370, 3256, 3020, 1734, 1698, 1675, 1255, 1164, 1040. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.1 (s, 1H), 8.81(s, 1H), 8.16 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.22 (d, J = 5.8 Hz, 2H), 7.06 (d, J = 5.8 Hz, 2H), 3.97 (s, 2H), 3.89 (s, 3H), 3.59 (tt, J = 7.2, 6.9 Hz, 1H), 3.47-3.44 (m, 4H), 2.84-2.82 (m, 4H), 1.36 (t, J = 6.9 Hz, 2H), 1.19 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 151.21 (d, J_{C-F} = 249.3 Hz), 146.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.85, 134.81, 133.86, 132.76, 131.69, 129.43, 128.11, 118.95 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.73, 56.21, 52.49, 51.77, 49.16, 34.72, 8.18. ESI-MS (m/z): calcd. for $C_{27}H_{27}FN_6O_4$ 518.21, found 519.29 $[M + H]^+$.

1-cyclopropyl-7-(4-((1-(3,4-dimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl) piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8m**)

Pale brown solid; yield: 50%, 0.29g, m.p. 108-110 °C; IR (KBr, cm^{-1}) 3365, 3250, 3025, 1730, 1695, 1670, 1250, 1175, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.12 (s, 1H), 8.71 (s, 1H), 8.17 (s, 1H), 7.94 (d, J_{H-F} = 13.2 Hz, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.08 (d, J = 5.8 Hz, 1H), 6.98 (d, J = 5.8 Hz, 1H), 6.23 (s, 1H), 3.97 (s, 2H), 3.78 (s, 6H), 3.59 (tt, J = 7.2, 6.9 Hz, 1H), 3.48-3.46 (m, 4H), 2.93-2.91 (m, 4H), 1.34 (t, J = 6.9 Hz, 2H), 1.19 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75

MHz, $CDCl_3$) δ 176.88 (d, J_{C-F} = 2.2 Hz), 166.43, 157.29, 151.25 (d, J_{C-F} = 249.3 Hz), 148.23, 145.82 (d, J_{C-F} = 10.3 Hz), 137.45, 135.21, 133.71, 131.89, 130.75, 127.43, 124.91, 118.95 (d, J_{C-F} = 8.1 Hz), 116.79, 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.78, 57.23, 52.57, 51.74, 49.19, 34.79, 8.25. ESI-MS (m/z): calcd. for $C_{28}H_{29}FN_6O_5$ 548.22, found 549.29 $[M + H]^+$.

7-(4-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8n**)

White solid; yield: 53%, 0.32g, m.p. 199-200 °C; IR (KBr, cm^{-1}) 3354, 3250, 3025, 1730, 1695, 1670, 1250, 1040, 585. ^1H NMR (300 MHz, $CDCl_3$) δ 15.13 (s, 1H), 8.82(s, 1H), 8.17 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.59 (d, *J* = 5.8 Hz, 2H), 7.48 (d, *J* = 5.8 Hz, 2H), 3.98 (s, 2H), 3.58 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.48-3.46 (m, 4H), 2.90-2.87 (m, 4H), 1.34 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.88 (d, J_{C-F} = 2.2 Hz), 166.33, 151.21 (d, J_{C-F} = 249.3 Hz), 146.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.75, 135.81, 134.86, 133.76, 131.69, 129.43, 128.15, 118.94 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.73, 52.49, 51.67, 49.18, 34.74, 8.19. ESI-MS (m/z): calcd. for $C_{26}H_{24}BrFN_6O_3$ 566.11, found 567.19 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-((1-(4-fluoro-2-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8o**)

Brown solid; yield: 60%, 0.41g, m.p. 232-233 °C; IR (KBr, cm^{-1}) 3360, 3250, 3025, 1730, 1695, 1670, 1250, 1045, 525. ^1H NMR (300 MHz, $CDCl_3$) δ 14.99 (s, 1H), 8.71 (s, 1H), 8.17 (s, 1H), 7.94 (d, J_{H-F} = 13.2 Hz, 1H), 7.73 (s, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.52 (d, *J* = 5.8 Hz, 1H), 7.16 (d, *J* = 5.8 Hz, 1H), 3.97 (s, 2H), 3.59 (tt, *J* = 7.2, 6.9 Hz, 1H), 3.47-3.43 (m, 4H), 2.84-2.82 (m, 4H), 1.38 (t, *J* = 6.9 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 157.19, 151.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.85, 134.81, 132.76, 131.79, 130.78, 127.43, 124.81, 118.95 (d, J_{C-F} = 8.1 Hz), 116.79, 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.76, 52.44, 51.78, 49.16, 34.64, 8.13. ESI-MS (m/z): calcd. for $C_{26}H_{23}F_2IN_6O_3$ 632.08, found 633.16 $[M + H]^+$.

1-cyclopropyl-7-(4-((1-(4-ethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8p**)

White solid; yield: 67%, 0.38g, m.p. 187-188 °C; IR (KBr, cm^{-1}) 3345, 3245, 3025, 1730, 1695, 1670, 1250, 1155, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.12 (s, 1H), 8.84(s, 1H), 8.19 (s, 1H), 7.97 (d, J_{H-F} = 13.2 Hz, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.27 (d, J = 5.8 Hz, 2H), 7.12 (d, J = 5.8 Hz, 2H), 3.97 (s, 2H), 3.91-3.89 (m, 2H), 3.59 (tt, J = 7.2, 6.9 Hz, 1H), 3.46-3.44 (m, 4H), 2.85-2.82 (m, 4H), 1.36 (t, J = 6.9 Hz, 2H), 1.31 (t, J = 6.9 Hz, 3H), 1.19 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.87 (d, J_{C-F} = 2.2 Hz), 166.43, 151.23 (d, J_{C-F} = 249.3 Hz), 146.33, 145.82 (d, J_{C-F} = 10.3 Hz), 136.82, 134.31, 133.56, 132.26, 131.62, 129.43, 128.11, 118.95 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.83, 65.62, 52.59, 51.87, 49.26, 34.74, 16.34, 8.18. ESI-MS (m/z): calcd. for $C_{28}H_{29}FN_6O_4$ 532.22, found 533.29 $[M + H]^+$.

1-cyclopropyl-7-(4-((1-(3,4-difluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8q**)

Pale yellow solid; yield: 73%, 0.41g, m.p. 118-119 °C; IR (KBr, cm^{-1}) 3364, 3252, 3025, 1730, 1694, 1670, 1250, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.09 (s, 1H), 8.74 (s, 1H), 8.19 (s, 1H), 7.95 (d, J_{H-F} = 13.2 Hz, 1H), 7.83 (s, 1H), 7.69 (d, J_{H-F} = 7.5 Hz, 1H), 7.62 (d, J = 5.8 Hz, 1H), 7.26 (d, J = 5.8 Hz, 1H), 3.98 (s, 2H), 3.59 (tt, J = 7.2, 6.9 Hz, 1H), 3.47-3.45 (m, 4H), 2.87-2.84 (m, 4H), 1.39 (t, J = 6.9 Hz, 2H), 1.19 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.83 (d, J_{C-F} = 2.2 Hz), 166.93, 157.39, 151.61 (d, J_{C-F} = 249.3 Hz), 147.53, 145.92 (d, J_{C-F} = 10.3 Hz), 136.95, 135.81, 133.76, 132.89, 130.95, 128.73, 124.91, 118.95 (d, J_{C-F} = 8.1 Hz), 116.89, 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.76, 52.97, 51.79, 49.19, 34.59, 8.16. ESI-MS (m/z): calcd. for $C_{26}H_{23}F_3N_6O_3$ 524.18, found 525.23 $[M + H]^+$.

7-(4-((1-(4-bromo-2-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8r**)

Pale brown solid; yield: 50%, 0.31g, m.p. 158-159 °C; IR (KBr, cm^{-1}) 3350, 3259, 3015, 1730, 1695, 1670, 1250, 1035, 610. ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.99 (s, 1H), 8.78 (s, 1H), 8.05 (d, J_{H-F} = 13.2 Hz, 1H), 7.77 (s, 1H), 7.67 (s, 1H), 7.57 (d, J_{H-F} = 7.5 Hz, 1H), 7.45 (d, J = 5.8 Hz, 1H), 7.26 (d, J = 5.8 Hz, 1H), 3.96 (s, 2H), 3.58 (tt, J = 7.2, 6.9 Hz, 1H), 3.49-3.47 (m, 4H),

2.85-2.82 (m, 4H), 1.86 (s, 3H), 1.41 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 157.19, 151.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.82 (d, J_{C-F} = 10.3 Hz), 137.65, 135.91, 133.76, 132.79, 130.78, 129.43, 124.81, 118.95 (d, J_{C-F} = 8.1 Hz), 116.79, 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.76, 52.46, 51.79, 49.26, 34.74, 16.31, 8.15. ESI-MS (m/z): calcd. for $C_{27}H_{26}BrFN_6O_3$ 580.12, found 581.19 $[M + H]^+$.

1-cyclopropyl-6-fluoro-7-(4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8s**)

Yellow solid; yield: 63%, 0.36g, m.p. 265-266 °C; IR (KBr, cm^{-1}) 3375, 3252, 3022, 1736, 1696, 1673, 1526, 1357, 1251, 1043. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.11 (s, 1H), 8.82 (s, 1H), 8.17 (s, 1H), 7.98 (d, J_{H-F} = 13.2 Hz, 1H), 7.68 (d, J_{H-F} = 7.5 Hz, 1H), 7.61 (d, J = 5.8 Hz, 2H), 7.52 (d, J = 5.8 Hz, 2H), 3.98 (s, 2H), 3.58 (tt, J = 7.2, 6.9 Hz, 1H), 3.50-3.47 (m, 4H), 2.89-2.86 (m, 4H), 1.36 (t, J = 6.9 Hz, 2H), 1.19 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.88 (d, J_{C-F} = 2.2 Hz), 166.25, 151.26 (d, J_{C-F} = 249.3 Hz), 147.83, 146.62 (d, J_{C-F} = 10.3 Hz), 137.75, 136.91, 135.86, 132.76, 130.69, 129.63, 125.15, 118.94 (d, J_{C-F} = 8.1 Hz), 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.73, 52.49, 51.67, 49.28, 36.74, 8.18. ESI-MS (m/z): calcd. for $C_{26}H_{24}FN_7O_5$ 533.18, found 534.26 $[M + H]^+$.

7-(4-((1-(4-bromo-3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8t**)

Pale yellow solid; yield: 82%, 0.56g, m.p. 146-147 °C; IR (KBr, cm^{-1}) 3365, 3252, 3025, 1734, 1695, 1670, 1250, 1045, 620. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.11 (s, 1H), 8.74 (s, 1H), 8.19 (s, 1H), 7.95 (d, J_{H-F} = 13.2 Hz, 1H), 7.89 (s, 1H), 7.69 (d, J_{H-F} = 7.5 Hz, 1H), 7.52 (d, J = 5.8 Hz, 1H), 7.34 (d, J = 5.8 Hz, 1H), 3.96 (s, 2H), 3.59 (tt, J = 7.2 Hz, J = 6.9 Hz, 1H), 3.47-3.45 (m, 4H), 2.87-2.84 (m, 4H), 1.39 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.83 (d, J_{C-F} = 2.2 Hz), 166.93, 157.39, 151.61 (d, J_{C-F} = 249.3 Hz), 147.53, 145.92 (d, J_{C-F} = 10.3 Hz), 136.95, 135.81, 133.76, 132.89, 130.95, 128.73, 124.91, 121.34, 118.95 (d, J_{C-F} = 8.1 Hz), 117.84, 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.76, 52.97, 51.76, 49.18, 34.59, 8.15. ESI-MS (m/z): calcd. for $C_{27}H_{23}BrF_4N_6O_3$ 634.1, found 635.18 $[M + H]^+$.

7-(4-((1-(benzo[d][1,3]dioxol-4-yl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8u**)

White solid; yield: 61%, 0.35g, m.p. 147-148 °C; IR (KBr, cm^{-1}) 3378, 3254, 3025, 1730, 1699, 1673, 1254, 1125, 1040. ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.99 (s, 1H), 8.67 (s, 1H), 7.9 (d, J_{H-F} = 13.2 Hz, 1H), 7.82 (s, 1H), 7.49 (d, J_{H-F} = 7.5 Hz, 1H), 7.29 (d, J = 5.8 Hz, 1H), 7.12 (dd, J = 5.8 Hz, 1H), 6.89 (d, J = 5.8 Hz, 1H), 6.03 (s, 2H), 3.86 (s, 2H), 3.57 (tt, J = 7.2, 6.9 Hz, 1H), 3.40-3.38 (m, 4H), 2.86-2.84 (m, 4H), 1.39 (t, J = 6.9 Hz, 2H), 1.19 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.83 (d, J_{C-F} = 2.2 Hz), 166.93, 157.39, 151.61 (d, J_{C-F} = 249.3 Hz), 147.53, 145.92 (d, J_{C-F} = 10.3 Hz), 138.95, 136.81, 134.86, 132.49, 130.95, 125.92, 121.33, 118.95 (d, J_{C-F} = 8.1 Hz), 117.84, 112.94 (d, J_{C-F} = 24.14 Hz), 109.87 (d, J_{C-F} = 3.7 Hz), 102.74, 101.02, 52.97, 51.75, 49.23, 34.49, 8.17. ESI-MS (m/z): calcd. for $C_{27}H_{25}FN_6O_5$ 532.19, found 533.28 $[M + H]^+$.

1-cyclopropyl-7-(4-((1-(2,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methyl) piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8v**)

Pale yellow solid; yield: 75%, 0.41g, m.p. 212-213 °C; IR (KBr, cm^{-1}) 3370, 3255, 3020, 1734, 1698, 1670, 1255, 1055. ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.99 (s, 1H), 8.69 (s, 1H), 8.12 (s, 1H), 7.94 (d, 1H, J_{H-F} = 13.2 Hz), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.51 (d, J = 5.8 Hz, 1H), 7.24 (d, J = 5.8 Hz, 1H), 7.12 (s, 1H), 3.96 (s, 2H), 3.58 (tt, J = 7.2, 6.9 Hz, 1H), 3.49-3.47 (m, 4H), 2.84-2.82 (m, 4H), 2.42 (s, 3H), 1.96 (s, 3H), 1.38 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 157.19, 151.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.25, 135.91, 134.26, 132.13, 131.74, 129.43, 126.41, 118.95 (d, J_{C-F} = 8.1 Hz), 116.79, 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.76, 52.46, 51.78, 49.26, 34.74, 22.13, 16.31, 8.15. ESI-MS (m/z): calcd. for $C_{28}H_{29}FN_6O_3$ 516.23, found 517.29 $[M + H]^+$.

1-cyclopropyl-7-(4-((1-(3,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8w**)

Pale yellow solid; yield: 83%, 0.50g, m.p. 175-176 °C; IR (KBr, cm^{-1}) 3364, 3255, 3025, 1730, 1693, 1675, 1254, 1040, 785. ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.07 (s, 1H), 8.69 (s, 1H), 8.19 (s,

1H), 7.95 (d, J_{H-F} = 13.2 Hz, 1H), 7.79 (s, 1H), 7.69 (d, J_{H-F} = 7.5 Hz, 1H), 7.59 (d, J = 5.8 Hz, 1H), 7.19 (d, J = 5.8 Hz, 1H), 3.97 (s, 2H), 3.59 (tt, J = 7.2, 6.9 Hz, 1H), 3.48-3.46 (m, 4H), 2.87-2.85 (m, 4H), 1.38 (t, J = 6.9 Hz, 2H), 1.19 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.83 (d, J_{C-F} = 2.2 Hz), 166.93, 157.39, 151.61 (d, J_{C-F} = 249.3 Hz), 147.53, 145.92 (d, J_{C-F} = 10.3 Hz), 136.95, 135.81, 133.76, 132.89, 130.95, 128.73, 124.91, 118.95 (d, J_{C-F} = 8.1 Hz), 116.89, 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.76, 52.97, 51.79, 49.19, 34.59, 8.16. ESI-MS (m/z): calcd. for $C_{26}H_{23}Cl_2FN_6O_3$ 556.12, found 557.21 $[M + H]^+$.

1-cyclopropyl-7-(4-((1-(2,4-difluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8x**)

Pale yellow solid; yield: 72%, 0.40g, m.p. 152-153 °C; IR (KBr, cm^{-1}) 3375, 3254, 3025, 1730, 1695, 1670, 1250, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.99 (s, 1H), 8.71 (s, 1H), 8.16 (s, 1H), 7.94 (d, J_{H-F} = 13.2 Hz, 1H), 7.79 (s, 1H), 7.67 (d, J_{H-F} = 7.5 Hz, 1H), 7.58 (d, J = 5.8 Hz, 1H), 7.26 (d, J = 5.8 Hz, 1H), 3.98 (s, 2H), 3.59 (tt, J = 7.2, 6.9 Hz, 1H), 3.47-3.44 (m, 4H), 2.85-2.82 (m, 4H), 1.39 (t, J = 6.9 Hz, 2H), 1.18 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.86 (d, J_{C-F} = 2.2 Hz), 166.33, 157.19, 151.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.82 (d, J_{C-F} = 10.3 Hz), 136.85, 134.81, 132.76, 131.79, 130.78, 127.43, 124.81, 118.95 (d, J_{C-F} = 8.1 Hz), 116.79, 112.91 (d, J_{C-F} = 24.14 Hz), 109.86 (d, J_{C-F} = 3.7 Hz), 102.76, 52.54, 51.77, 49.11, 34.65, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{23}F_3N_6O_3$ 524.18, found 525.26 $[M + H]^+$.

3.4.2. Biological Assay

3.4.2.1. Cell Culture

Human caucasian acute lymphoblastic leukaemia cells (CCRF-CEM), breast adenocarcinoma (MDA-MB-468) and human colon carcinoma cells (HCT-116) were obtained from American type culture collection. Ovarian carcinoma cell line (SK-OV-3) and human T cell lymphoblast cell line (CCRF-CEM) were obtained from American type culture collection. Cells were grown on 75 cm^2 cell culture flasks with EMEM (Eagle's minimum essential medium), supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin solution (10,000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO_2, 95% air at 37 °C.

3.4.2.2. Cell Proliferation assay

Cell proliferation assay was carried out using Cell Titer 96 aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, upon reaching about 75-80% confluency, 5000 cells/well were plated in 96-well microplate in 100 EL media. After seeding for 72 h, the cells were treated with 50 µM and 10 µM compound in triplicate. Doxorubicin (Dox) (10 µM) was used as the positive control. At the end of the sample exposure period (72 h), 20 µL Cell Titer 96 aqueous solution was added. The plate was returned to the incubator for 1 h in a humidified atmosphere at 37 °C. The absorbance of the formazan product was measured at 490 nm using microplate reader. The blank control was recorded by measuring the absorbance at 490 nm with wells containing medium mixed with Cell Titer 96 aqueous solution but no cells. Results were expressed as the percentage of the control (without compound set at 100%).

3.4.2.3. MTT assay

Cell viability was determined by (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [41]. Human lung cancer cell line (A549), human cervical cancer cell line (HeLa), Human Breast carcinoma cell lines MDA MB-231, MCF7, Human Pancreatic Cancer line MiaPaca-2 and Human Embryonic Kidney Cell lines (HEK) were employed in current study. Cells (1×10^4 cells/well) were seeded to 96-well culture plate and cultured with or without different concentrations compounds for 48h in a final volume of 200 µL. After treatment, the medium was removed and 10 µL of MTT (10 mg/mL in PBS) was added to the fresh medium. After 2 h incubation at 37 °C, 100 µL extraction buffer was added to each well and plates were agitated for 1 min. The optical density (O.D) was read at 570nm using micro plate reader (Multimode Varioskan Flash Instrument-Thermo Scientific Ltd). Percent inhibition of proliferation was calculated as a fraction of control (without compound). All the experiments were carried out in triplicates. The results were represented as percentage of cytotoxity/viability. From the percentage of cytotoxicity the IC_{50} values are calculated.

3.4.3. Docking studies:

Further the molecular docking studies of were performed using human topo-II isomerase, α, β-tubulin subunits using Schrödinger suite 2013. Crystal co-ordinates for DNA topo-II isomerase, α, β-tubulin subunits were taken from Protein Data Bank (PDB ID: 4G0U, 3E22) [42]. The multi-step Schrödinger's protein preparation tool (PPrep) has been used for final preparation of receptor model. Hydrogens were added to the model automatically via the maestro interface. PPrep neutralizes side chains and residues which are not involving in salt bridges. This step is then followed by restrained minimization using the OPLS 2005 force field to RMSD of 0.3 A°. The 2D structure were sketched and converted to 3D using maestro interface. Ligands were prepared for docking using Ligprep, module of Schrodinger [43]. A total of 10 conformations were generated for all the compounds. Grid box was generated with considering co-crystal ligand i.e. amsacrine and colchicine. Docking studies were performed using GLIDE, module of Schrödinger. Docking scores by standard precision (Glide-SP) [44] docking were shown in Tables. Amino acid interaction pattern of few active compounds were shown in Figures along with amsacrine and colchicine.

3.4.4. UV- Visible measurement:

The DNA binding experiments were carried in Tris–HCl buffer solution (5 mM, pH 7.4) using the compound solution in DMSO. In UV–visible measurements, a constant concentration of compound **6o** and **8t** was treated with different concentrations of the CtDNA. The DNA solutions of equivalent concentrations were measure as reference solutions in experiment. The absorbance (A) was recorded after successive additions of various concentrations of CtDNA. An equal amount of CtDNA was added to the compound solution and the reference solution while measuring the spectra to eliminate the absorbance of the CtDNA itself.

3.4.5. Fluorescence measurements:

The fluorescence emission spectra were measured at 300 K over a wavelength range of 520−740 nm with an exciting wavelength at 500 nm. Before measuring fluorescence spectra, all solutions were stirred and allowed to equilibrate for 5 min. For correct background fluorescence blank of Tris–HCl buffer was subtracted.

3.5. References:

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Chapter IV

Design and synthesis of 2-(4-aminophenyl)benzothiazole analogues as antiproliferative agents

Chapter 4

Design and synthesis of 2-(4-aminophenyl)benzothiazole analogues as antiproliferative agents

4.1. Introduction

Benzothiazole is a fused bicyclic system, and is well known nucleus in anticancer research. Benzothiazoles exhibit interesting pharmacological activities such as anti-inflammatory, antiallergic, antitumor and analgesic activities [1-7]. Many modifications happened on benzothiazole moiety and several research groups evaluated them for various biological activities. Among all the benzothiazole derivatives, 2-(4-aminophenyl)benzothiazoles, are a novel class of potent and selective antitumor agents. 2-(4-aminophenyl)benzothiazoles are potent and active in certain human breast cancer cell lines both *in vitro* and *in vivo* [8]. 2-(4-amino-3-methylphenyl)-5-fluorobenzthiazole is presently in phase I clinical trial in UK. It exhibits antitumor activity by binding to the arylhydrocarbon receptor (AhR) and translocates into the nucleus, induction of the cytochrome P450 isoform (CYP) 1A1. This converts the drug into reactive metabolites and forms DNA adducts causing cell death [9]. Among the benzothiazole derivatives structurally related benzothiazole such as 2-(4-amino-3-methylphenyl) benzothiazole (DF203), 2-(4-amino-3-methylphenyl)-5-fluorobenzthiazole (5F203) are clinical trial moiety phortress and 2-(3,4-dimethoxy phenyl)-5-fluorobenzothiazole (PMX610) exhibited notable *in vitro* antitumor activity against malignant cell lines [10]. Benzothiazole based anticancer agents are shown in **Figure 4.1**.

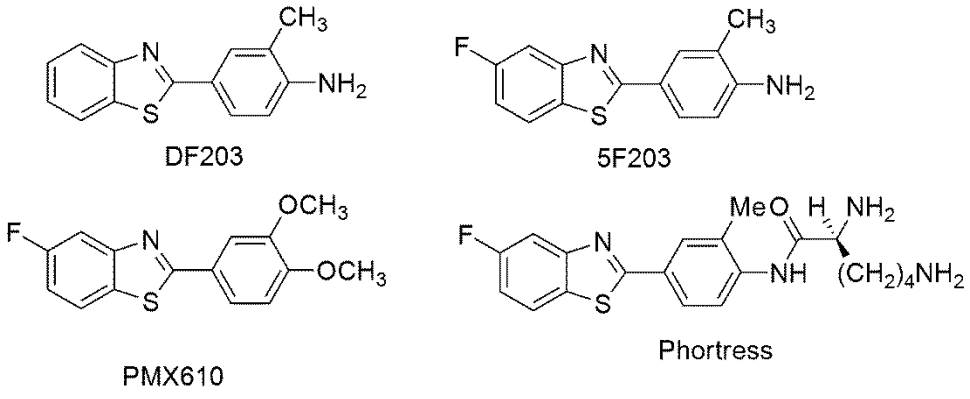


Figure 4.1: Benzothiazole based anticancer agents

Piperazine is a prominent six membered, nitrogen containing heterocycle of noteworthy significance in medicinal chemistry [11]. Piperazine analogues are reported to elicit a wide range of pharmacological activities such as antidepressant [12], anticancer [13], anthelmentic [14], antibacterial [15], antifungal [16], antimycobacterial [17], antimalarial [18], anticonvulsant [19]. The existence of this piperazines heterocycle can be witnessed in several identified drugs, belonging to different pharmacological classes [20]. Several researchers have developed piperazine based anticancer agents. Nagarapu *et al.*, synthesized piperazine containing (*R*/*S*)-2-[2-hydroxy-3-(4-phenylpiperazin-1-yl)propyl]-1*H*-pyrrolo[3,4-*b*]quinolin-3(2*H*)-one derivatives, these analogues were screened for anticancer activity against SK-N-SH and A549 cell lines *in vitro*. Among all the derivatives, compound **F** (Figure 4.2) was reported as the most effective inhibitor [21]. Shallal *et al.*, synthesized piperazinylpyrimidine derivatives, and screened for anticancer activity. Compound **G** (Figure 4.2) emerged as the most potent growth inhibitor of MDA-MB-468 cell line [22]. Lin *et al.*, developed piperazine substituted derivatives and screened for antiproliferative activity against three cell lines. Compound **H** (Figure 4.2), which elicited IC_{50} values of 7.34, 10.39 and 3.49 μM against A549, MCF-7 and HCT-116 cells was reported as the most active compound of the series [23]. Fytas *et al.*, synthesized different piperazine derivatives, amongst compound **I** (Figure 4.2) was found to be most active against HeLa and MDA MB 231 cancer cell lines respectively with 8.4 and 6.8 μM IC_{50} values [24]. Piperazine based anticancer analogues are depicted in **Figure 4.2**.

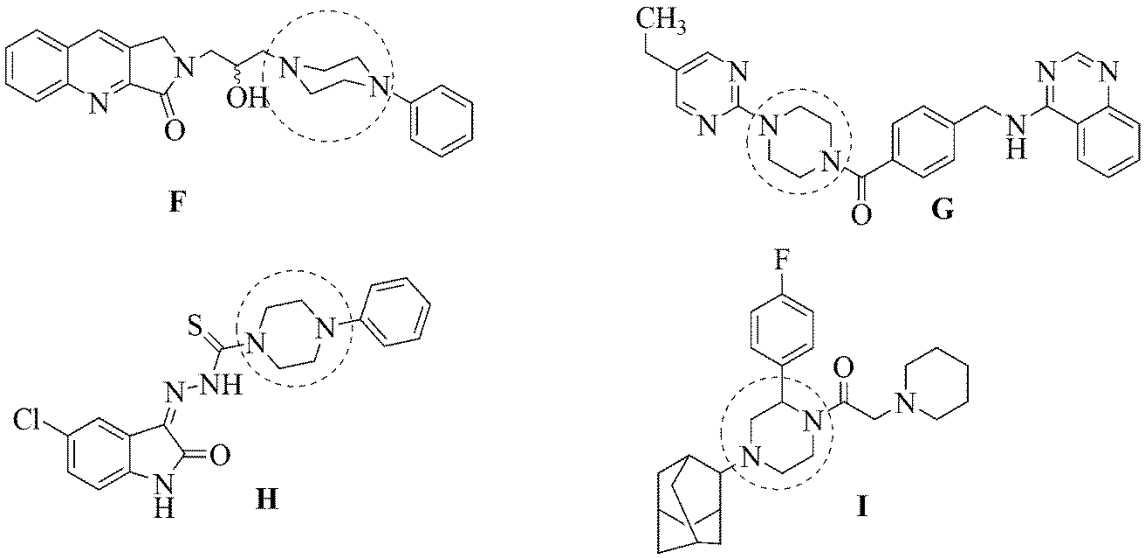


Figure 4.2: Anticancer compounds based on piperazine.

From the past few years, 1,2,3-triazole derivatives have been synthesized as constructive chemotherapeutic agents for different diseases [25]. 1,2,3-triazole derivatives have significant position in medicinal chemistry due to their easy synthesis by click chemistry and attractive biological activities, such as antibiotic, antifungal, antehelmintic [26-29], and anticancer activity in different human cancer cell lines [30-33]. For development of new anticancer agents, the 1,2,3-triazoles with other pharmacophores via click chemistry, with potent anticancer activity were synthesized. For example, novel 1,2,3-triazole-pyrimidine hybrids were synthesized and evaluated for their anticancer activity. Most of the synthesized compounds exhibited moderate to good activity against MGC-803, EC-109, MCF-7 and B16-F10 cancer cell lines [34]. A series of novel 1,2,3-triazole-dithiocarbamate hybrids were designed, synthesized and evaluated for anticancer activity against MGC-803, MCF-7, PC-3, EC-109 human tumor cell lines, they exhibited moderate to potent activity against MGC-803 and MCF-7 cell line [35]. A series of 1,2,3-triazole bearing podophyllotoxins were synthesized and evaluated for anticancer activity against SF-295, A-549, PC-3, Hep-2, HCT-15 and MCF-7 cell lines, majority of the compounds proved to be more potent than etoposide and compounds were exhibited significant anticancer activity with IC_{50} values in the range of 0.001-1 μM [36].

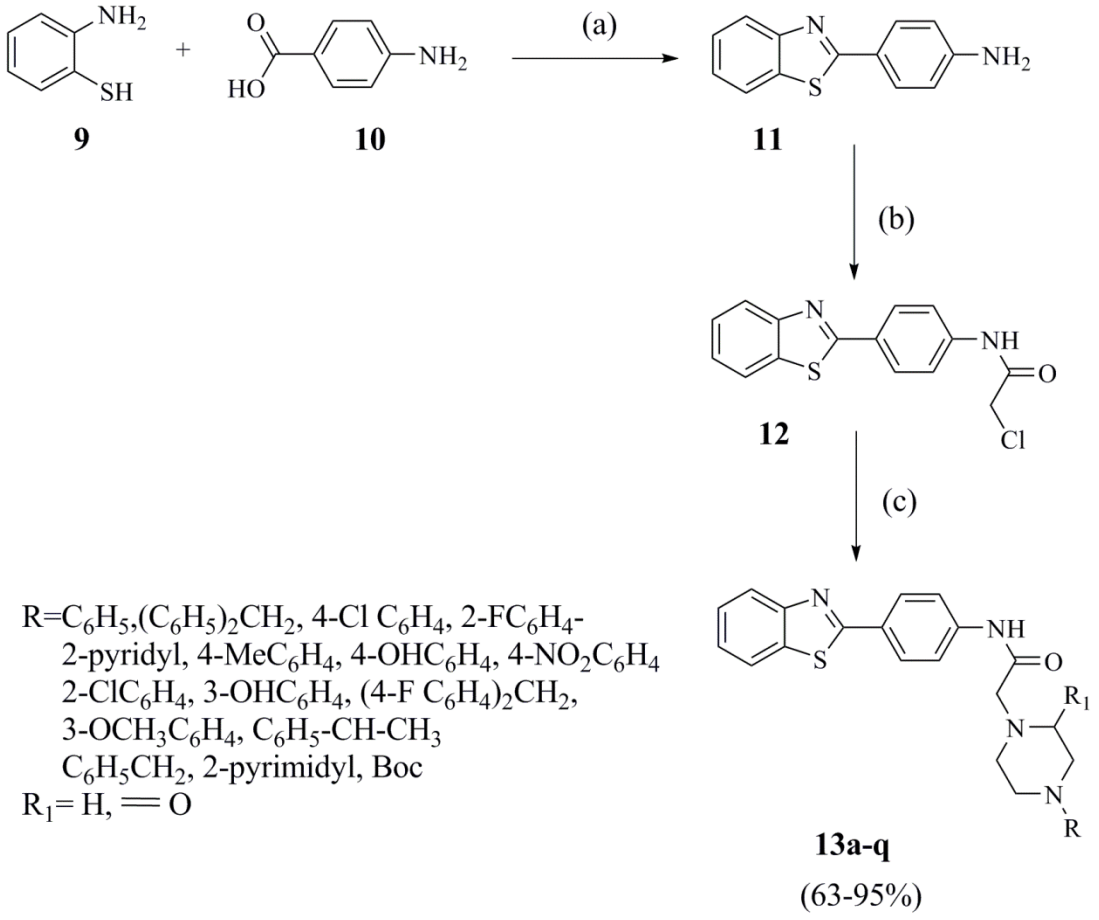
Molecular hybridization is the rational design of new chemical entities by the combination of two or more active compounds or pharmacophoric units recognized and derived from known bioactive molecules [37, 38]. In persistence of our continuing efforts on the design of novel anticancer agents and realizing the importance of benzothiazoles, piperazines, 1,2,3-triazole and their derivatives in chemotherapy, we designed based on hybridization approach and synthesized novel 2-(4-aminophenyl)benzothiazole derivatives and performed antiproliferative evaluation on a panel of three cancer cell lines A549 (Lung cancer), HeLa (Cervical cancer) and MDA-MB-231 (Breast cancer).

4.2. Results and Discussion

4.2.1. Chemistry

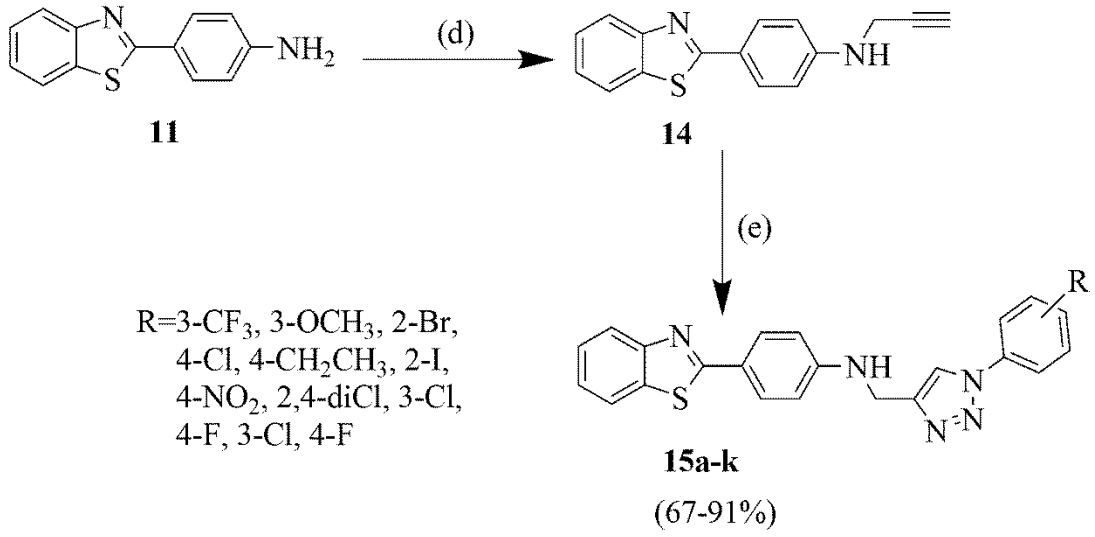
The synthetic strategies for the synthesis of the intermediates and title compounds are depicted in **scheme 4** and **scheme 5**. We synthesized 2-(4-aminophenyl)benzothiazole (**11**) by the reaction of 2-aminothiophenol (**9**) with *p*-amino benzoic acid (**10**) in polyphosphoric acid at 220 °C as per reported procedure [39]. The IR spectrum of compound **11** showed absorption bands at 3290,

3385 cm^{-1} due to asymmetric and symmetric stretching of NH_2, and a peak at 1670 cm^{-1} due to C=N stretching vibrations and mass spectrum revealed a (m+1) peak at m/z = 227.09 of 2-(4-aminophenyl)benzothiazole. 2-(4-aminophenyl)benzothiazole (**11**) was treated with chloroacetyl chloride in the presence of triethylamine at 0 °C to yield *N*-(4-(benzo[*d*]thiazol-2-yl)phenyl)-2-chloroacetamide (**12**) as product. The IR spectrum of compound **12** showed absorption band at 1650 cm^{-1} due to carbonyl group of carboxamide and mass spectrum revealed a (m+1) peak at m/z = 303.11 of *N*-(4-(benzo[*d*]thiazol-2-yl)phenyl)-2-chloroacetamide. *N*-(4-(benzo[*d*]thiazol-2-yl)phenyl)-2-chloroacetamide (**12**) on treatment with different substituted piperazines in *N*,*N*-dimethylformamide at 100 °C for 2h yielded the products **13a-q**.



Scheme 4: Reagents and conditions (a) polyphosphoric acid, 220 °C (b) chloroacetyl chloride, Et_3N, 0 °C-RT (c) substituted piperazines, 100 °C, 2h

2-(4-aminophenyl)benzothiazole (**11**) on treatment with propargyl bromide yielded 4-(benzo[*d*]thiazol-2-yl)-N-(prop-2-ynyl)aniline (**14**) in 80% yield after purification. The IR spectrum of compound **14** showed absorption bands at 2132, 3325 cm^{-1} due to C≡C & ≡C−H stretching and mass spectrum revealed a (m+1) peak at m/z = 265.15 of 4-(benzo[*d*]thiazol-2-yl)-*N*-(prop-2-ynyl)aniline. 4-(benzo[*d*]thiazol-2-yl)-*N*-(prop-2-ynyl)aniline (**14**) on treatment with various aromatic azides in the presence of $CuSO_4.5H_2O$, sodium ascorbate in *t*BuOH:H_2O (1:1) gave the final products **15a-k** in 55-90% yields.



Scheme 5: Reagents and conditions: (d) propargyl bromide, K_2CO_3, DMF, 70 °C, 1h (e) various aromatic azides, $CuSO_4.5H_2O$, sodium ascorbate, *t*BuOH:H_2O (1:1), 2h

In general, ^{1}H NMR of all the title compounds displayed two multiplets of piperazine protons resonated in the range 2.5-3.7 ppm. Two sharp doublets resonated in the range 7.50-7.90 ppm due to C-5 and C-6 protons of the benzothiazole moiety. Two sharp doublets resonated in the range 8.10-8.20 ppm due to C-4 and C-7 protons of the benzothiazole moiety. Two sharp doublets resonated in the range 7.81-7.97 ppm due to phenyl protons. The acetyl link protons showed singlet in the range 3.29-3.39 ppm and further, the structure of the title compounds were substantiated from ^{13}C NMR and ESI MS respectively. All the compounds were evaluated for their antiproliferative activity and the results are summarized in **Table 4.1**.

4.2.2. Antiproliferative activity

In vitro antiproliferative activity of the synthesized compounds **13a-q** and **15a-k** was carried out against three types of human cancer cell lines; A549 (lung cancer), HeLa (cervical cancer) and MDA-MB-231(breast cancer) employing sulforhodamine B (SRB) assay method [40, 41]. The growth inhibition data (expressed as GI_{50}) of synthesized compounds **13a-q** and **15a-k** are shown in **Table 4.1**.

Table 4.1: Antiproliferative activity ($^{a}GI_{50}$ μM) of compounds (**13a-q and 15a-k)**

Entry	R	R_1	A549	HeLa	MDA- MB-231	Docking Score (SP)
13a	C_6H_5	H	0.89±0.03	0.56±0.02	0.84±0.02	--
13b	$(C_6H_5)_2CH_2$	H	2.07±0.09	0.92±0.01	1.65±0.07	--
13c	4-Cl C_6H_4	H	1.02±0.09	0.98±0.04	1.0±0.01	--
13d	2-F C_6H_4-	H	0.96±0.02	1.0±0.09	0.45±0.02	--
13e	2-pyridyl	H	0.52±0.01	1.0±0.08	1.69±0.08	-3.747
13f	4-MeC_6H_4	H	**0.25±0.02**	0.81±0.01	0.95±0.02	--
13g	4-OHC_6H_4	H	**0.18±0.03**	0.86±0.02	6.9±0.24	-5.523
13h	4-$NO_2C_6H_4$	H	1.36±0.07	1.03±0.07	1.38±0.04	-6.455
13i	2-ClC_6H_4	H	0.78±0.03	0.84±0.02	1.23±0.02	--
13j	3-OHC_6H_4	H	**0.14±0.02**	0.83±0.01	8.3±0.16	--
13k	$(4\text{-F } C_6H_4)_2CH_2$	H	4.58±0.23	0.8±0.01	6.73±0.20	--
13l	3-$OCH_3C_6H_4$	H	0.52±0.01	1.07±0.8	14.1±0.7	-4.707
13m	C_6H_5-CH-CH_3	H	0.82±0.01	0.95±0.03	1.4±0.17	--
13n	$C_6H_5CH_2$	H	12.3±0.45	0.41±0.01	4.9±0.02	--
13o	2-pyrimidyl	H	6.54±0.6	0.94±0.02	4.1±0.08	-3.982
13p	Boc	O	6.28±0.1	0.86±0.04	1.23±0.17	--
13q	Boc	H	6.75±0.09	0.81±0.01	14.3±1.9	--
15a	3-CF_3	-	0.49±0.02	0.96±0.02	7.2±0.06	--
15b	3-OCH_3	-	0.55±0.02	0.98±0.05	1.7±0.06	-6.608
15c	2-Br	-	0.81±0.01	1.03±0.08	4.6±0.23	--

15d	4-Cl	-	0.71±0.01	1.09±0.06	10.4±0.87	-3.974
15e	4-CH_2CH_3	-	0.83±0.02	1.4±0.12	1.4±0.07	--
15f	2-I	-	0.49±0.04	1.8±0.04	2.8±0.2	--
15g	4-NO_2	-	1.24±0.08	0.99±0.05	5.6±0.32	-5.546
15h	2,4-diCl	-	2.53±0.09	0.91±0.01	3.5±0.16	--
15i	3-Cl,4-F	-	2.42±0.12	0.8±0.03	2.8±0.18	-4.475
15j	3-Cl	-	0.45±0.03	1.0±0.09	0.56±0.03	--
15k	4-F	-	4.2±0.15	1.2±0.05	**0.14±0.02**	--
Doxorubicin			<0.01	0.09±0.001	<0.01	
Paclitaxel			<0.01	0.023±0.002	<0.01	

[a]GI_{50}: 50% Growth inhibition

The SRB assay appeared to be more responsive than MTT assay, with better linearity with cell number and higher reproducibility [42, 43]. From the antiproliferative activity results, all the synthesized compounds showed comparable antiproliferative activity with GI_{50} values ranging from 0.18-14.3μM. The structure activity relationship of 2-(4-aminophenyl)benzothiazole derivatives reveal, that compounds with electron donating groups (EDG) at para position like methyl (**13f**), hydroxy (**13g**) exhibit good activity than compounds having electron withdrawing groups (EWG) like nitro (**13h**), chloro (**13c**) against A549 cell line. Pyridine containing derivative (**13e**) exhibited better activity than phenyl (**13a**), benzyl (**13n**) and pyrimidine (**13o**) derivatives against A549 cell line.

The fluoro substitution (**13d**) at ortho position replaced with chloro (**13i**) enhanced the antiproliferative activity. The hydroxy group at 3rd position (**13j**) or 4th position (**13g**) on phenyl ring has no impact on the activity against A549 cell line. Replacement of methoxy group with hydroxy group at meta position enhanced the activity in A549 cell line. The chloro substitution at meta position on phenyl of triazole derivative (**15j**) showed better activity (0.45μM) than other chloro derivatives like *p*-chloro (**15d**), 3-chloro,4-fluoro (**15i**) and 2,4-dichloro (**15h**) derivatives against A549 cell line.

2-(4-aminophenyl)benzothiazole analogs exhibited moderate activity against HeLa cancer cell line. 2-(4-aminophenyl)benzothiazole piperazine analogs (**13a-q**) exhibited better

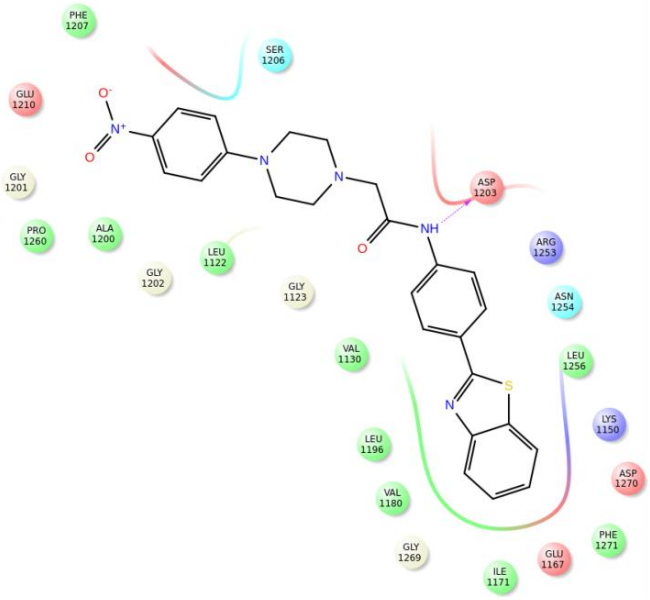
activity than 2-(4-aminophenyl)benzothiazole triazole derives (**15a-k**) against HeLa cancer cell line.

2-(4-aminophenyl)benzothiazole triazole derivatives (**15a-k**) exhibited better activity than 2-(4-aminophenyl)benzothiazole piperazine analogs (**13a-q**) against MDA-MB-231 cancer cell line. Among all the derivatives *p*-fluoro containing triazole derivative (**15k**) exhibited better activity (0.14 μM). *p*-fluoro containing triazole derivative (**15k**) showed better growth inhibition than compounds with electron withdrawing groups like *p*-nitro (**15g**), *p*-chloro (**15d**) and electron donating group like *p*-ethyl (**15e**) against MDA-MB-231 cancer cell line. Compared with 2,4-dichloro analog (**15h**) and 3-chloro,4-fluoro analog (**15i**), 4-fluoro analog exhibited better activity against MDA-MB-231 cancer cell line. *m*-chloro (**15j**) analog had better activity than *m*-trifluo methyl (**15a**) and *m*-methoxy (**15b**) analogs against MDA-MB-231 cancer cell line. Ortho substituted analogs (**15c**, **15f**) and disubstituted analogs (**15h**, **15i**) exhibited moderate activity against MDA-MB-231 cancer cell line.

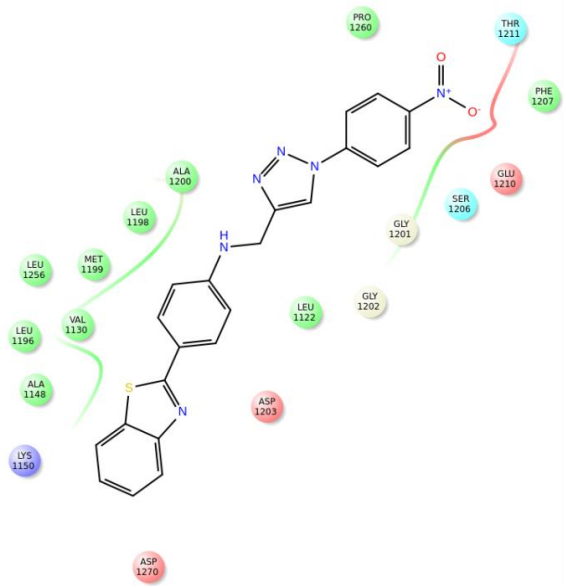
4.2.3. Molecular docking studies

The molecular docking studies of **13a-q** and **15a-k** were performed as a target of ALK (Human anaplastic lymphoma kinase) enzyme using Schrödinger suite 2013. Crystal co-ordinates for ALK (Human anaplastic lymphoma kinase) were taken from Protein Data Bank (PDB ID: 2XP2). Docking studies were performed using GLIDE, module of Schrödinger. Docking scores by standard precision (Glide-SP) docking were shown in **Table 4.1**.

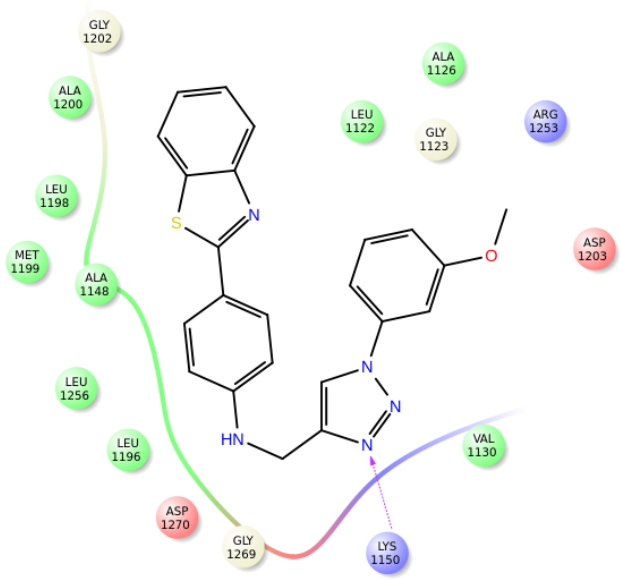
Molecular docking studies revealed that these compounds (**15b**, **13h** and **15g**) bind to the crizotinib binding site of the human anaplastic lymphoma kinase with a binding affinity of -6.608, -6.455 and -5.546, respectively, compared to crizotinib -8.123). This orientation is fruitful for extensive interactions such as hydrophobic interactions. Therefore, substitution with methoxy and nitro groups in **15b, 13h** and **15g** resulted in improved docking score, which contributed for the antiproliferative activity. Amino acid interaction pattern of few compounds **15b, 13h**, and **15g** are shown in **Figure 4.3**.



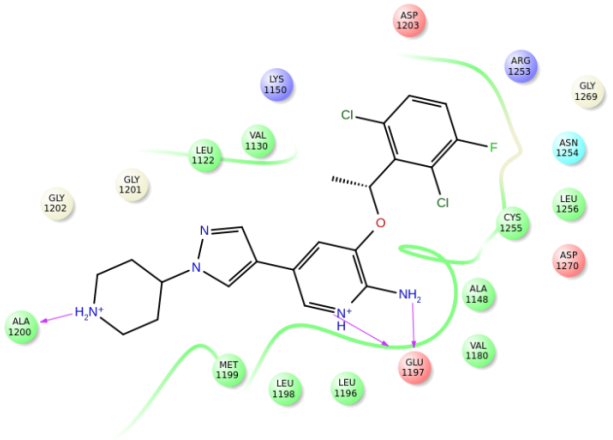
Compound 13h



Compound 15g

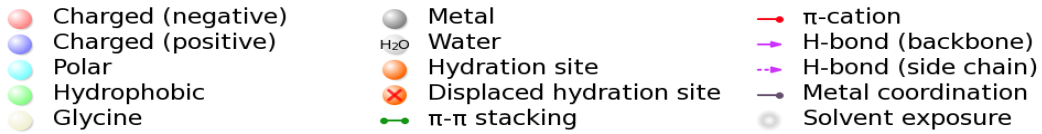


Compound 15b



Crizotinib

Figure 4.3: Amino acid interaction pattern of **13h, 15g, 15b** and **crizotinib**



4.3. Conclusion

In summary, a series of 2-(4-aminophenyl)benzothiazole analogues have been designed and synthesized, subsequently by easy reaction protocols. All the synthesized compounds were screened for their growth inhibitory activity against a panel of three different human cancer cell lines such as A549, HeLa and MDA-MB-231. Most of the tested 2-(4-aminophenyl)benzothiazole analogs displayed promising growth inhibitory activity against cancer cell lines. Among all the synthesized compounds, **13f, 13g,** and **15k** showed maximum growth inhibitory activity against cancer cell lines at low concentrations. Our findings from this work with synthesis, antiproliferative activity and molecular modeling experiments demonstrate that these 2-(4-aminophenyl)benzothiazole analogues could be potential candidates for developing novel anticancer agents.

4.4. Experimental section

4.4.1. Chemistry

All reagents were purchased from commercial sources and used with further purification wherever necessary. All reactions were monitored by analytical thin layer chromatography (TLC) performed on E-Merck 0.25 mm pre coated silica gel aluminum plates (60 F254) using mixture of pet ether and ethyl acetate. Visualization of the spots on TLC plates was achieved by exposure to UV light. Column chromatography was performed using silica gel (Acme, 100-200mesh). Solvents were dried and purified by distillation prior to use. Solvents for chromatography (Pet ether and ethyl acetate) were distilled prior to use. Evaporations were carried out under reduced pressure on Heidolf rotary evaporator. Melting points were obtained using Stuart SMP30 system and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Avance-III 400MHz (400 MHz for ^{1}H, 100 MHz for ^{13}C), in $CDCl_3$ or DMSO-d_6. Chemical shifts have been expressed in parts per million (δ) relative to tetramethylsilane (δ = 0.0) as an internal standard and coupling constants (J) in Hertz. Low-resolution mass spectra (LC-MS) were recorded on LC/MS-2020 Shimadzu. IR spectra were recorded with an FT-IR spectrophotometer (Jasco FTIR-4200).

Synthesis of 2-(4-aminophenyl)benzothiazole (**11**)

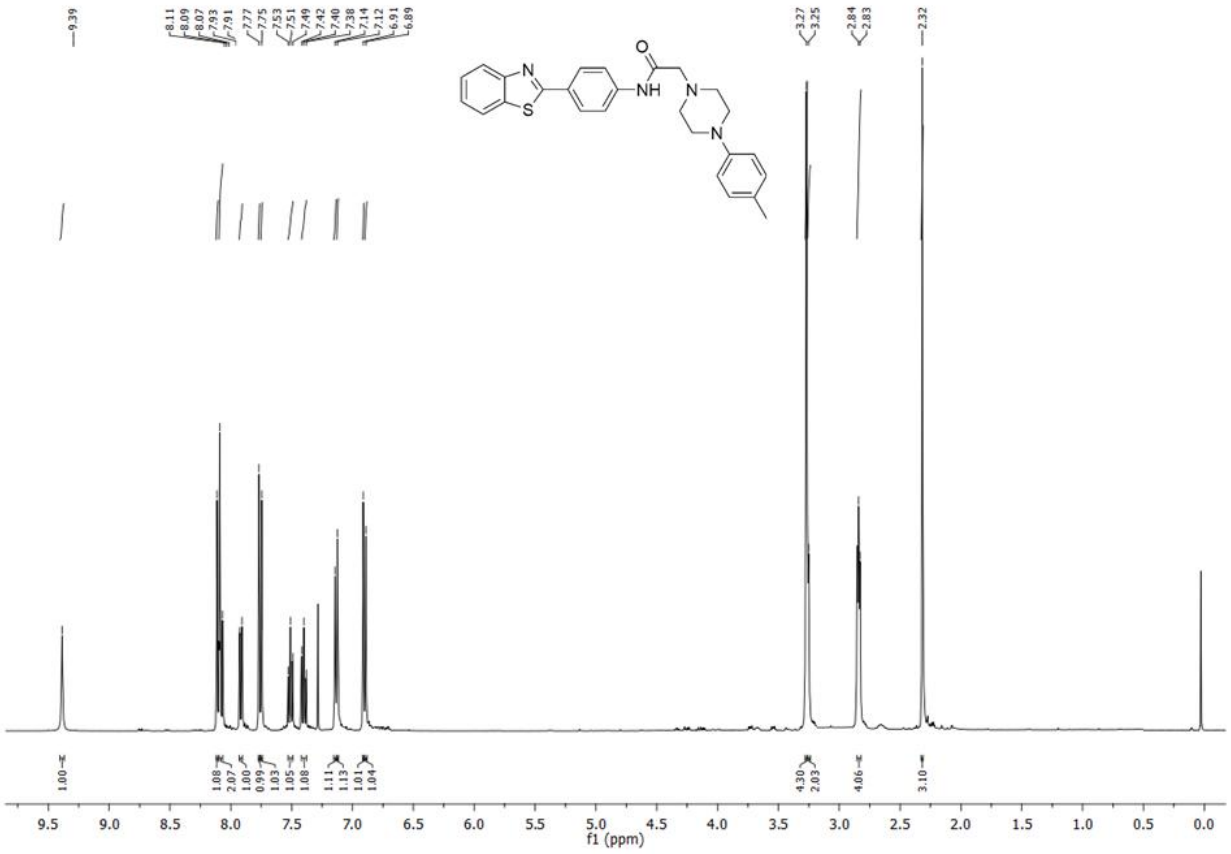
Synthesis of 2-(4-aminophenyl)benzothiazole (**11)** by the reported procedure [39]. 4-aminobenzoic acid (1g, 0.0072mol) (**10**) was dissolved in polyphosphoric acid at 220 °C. 2-Aminothiophenol (0.9g, 0.0072mol) was added and the resulting solution stirred at 220 °C for 30 min. After cooling, the reaction mixture was poured into aqueous ammonia (10 mL). The precipitate was collected and washed with water (50 mL). The product was purified by column chromatography (pet ether/ethylacetate 30%) to give the 2-(4-aminophenyl)benzothiazole (**11**) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (d, *J* = 7.25 Hz, 1H), 7.93 (d, *J* = 7.25 Hz, 1H), 7.70 (dd, *J* = 11.0, 2.0 Hz, 1H), 7.68 (dd, *J* = 10.9, 2.1 Hz, 1H), 7.63 (dd, *J* = 8.25, 2 Hz, 1H), 7.48 (t, *J* = 7.25 Hz, 1H), 7.46 (t, *J* = 7.25 Hz, 1H), 6.87 (t, *J* = 8.75 Hz, 1H), 5.98 (s, 2H). ^{13}C NMR (100.61 MHz, DMSO-d_6) 167.21, 154.65, 149.34, 135.56, 133.02, 127.54, 125.65, 123.23, 122.65, 120.85, 118.35. LCMS(m/z): calcd. for $C_{13}H_{10}N_2S$ 226.06. found 227.12 $[M + H]^+$.

Synthesis of N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-chloroacetamide **(12)**

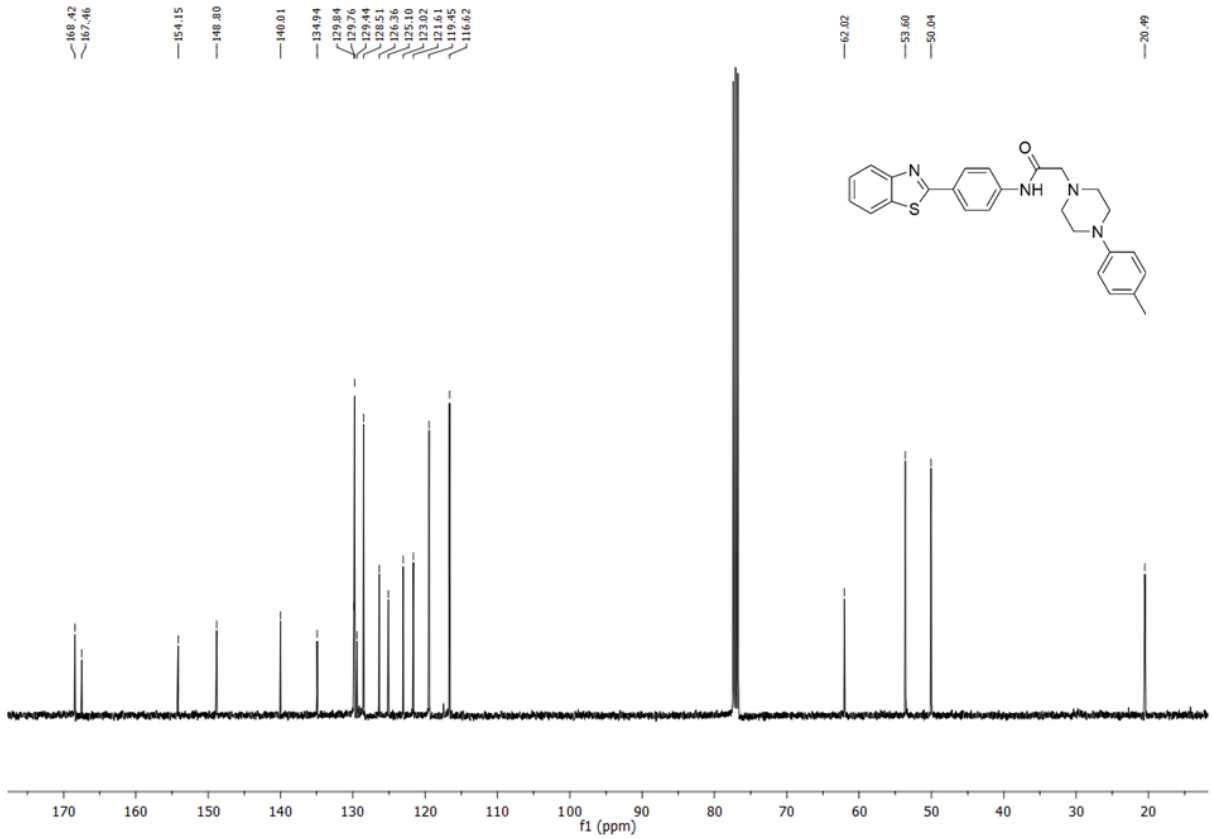
2-(4-aminophenyl)benzothiazole (**11**) (0.5g, 0.0022 mmol) and triethylamine (0.31 mL, 0.0022 mmol) were stirred in 5mL of dry dichloromethane (CH_2Cl_2) at 0 °C for 15 min under nitrogen (N_2) atmosphere. Chloroacetyl chloride (0.17 mL, 0.0022 mmol) was added drop wise slowly through a syringe. After stirring at 0 °C for 15 min, resultant mixture was warmed to room temperature (RT) and stirred for additional 1h. After the reaction was complete as indicated by TLC, 50ml of water was added and the compound was extracted from aqueous layer with 3×10 mL of CH_2Cl_2. The organic layers were collected, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield the desired product as a pale green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.04 (d, *J* = 7.25 Hz, 1H), 7.93 (d, *J* = 7.25 Hz, 1H), 7.70 (dd, *J* = 11.0, 2.0 Hz, 1H), 7.68 (dd, *J* = 10.9, 2.1 Hz, 1H), 7.63 (dd, *J* = 8.25, 2 Hz, 1H), 7.48 (t, *J* = 7.25 Hz, 1H), 7.46 (t, *J* = 7.25 Hz, 1H), 6.87 (t, *J* = 8.75 Hz, 1H), 3.25 (s, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 167.87, 154.12, 149.34, 135.21, 133.01, 127.32, 125.81, 124.11, 123.51, 122.02, 120.21, 118.32, 83.87. LCMS(m/z): calcd. for $C_{15}H_{11}ClN_2OS$ 302.02, found 302.09 $[M + H]^+$.

General procedure for synthesis of N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(2,4-substituted piperazin-1-yl)acetamide **(13a-q)**

To a solution of substituted piperazines (0.9933 mmol) in dry DMF (3mL), was added triethylamine (0.13mL, 1.9866 mmol) and potassium iodide (16.48mg, 0.0993 mmol) at RT under N_2 atmosphere. Then compound **12** (0.3g, 0.9933 mmol) was added and resultant mixture was heated for 3h at 125 °C. After the reaction was complete as indicated by TLC, DMF was evaporated under reduced pressure. The obtained residue was diluted with 30 mL of water. The compound was extracted from aqueous layer with 3×5 mL of CH_2Cl_2. The organic layers were collected, dried over anhydrous $MgSO_4$ and evaporated. The resultant residue was purified by column chromatography (pet ether/ethyl acetate 30%).



^{1}H NMR spectrum of compound **13f**



^{13}C NMR spectrum of compound **13f**

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-phenylpiperazin-1-yl)acetamide (**13a**)

Brown solid; yield 95%, 0.4g, m.p. 195-197 °C; IR (KBr, cm^{-1}) 3465, 3046, 1645, 1292, 1135. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (s, 1H), 8.09 (d, J = 1.8 Hz, 1H), 8.12 – 8.07 (m, 2H), 7.95 – 7.91 (m, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.71 (d, J = 2.0 Hz, 2H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.15 – 7.14 (m, 1H), 7.12 (d, *J* = 0.8 Hz, 1H), 6.91 (d, J = 2.1 Hz, 2H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.83, 168.26, 154.93, 149.25, 138.12, 133.62, 129.61, 129.01, 126.32, 125.11, 124.82, 121.98, 121.34, 121.01, 119.86, 114.42, 61.15, 51.34, 49.58, LCMS(m/z): calcd. for $C_{25}H_{24}N_4OS$ 428.17. found 429.19 $[M + H]^+$.

2-(4-benzhydrylpiperazin-1-yl)-N-(4-(benzo[d]thiazol-2-yl)phenyl)acetamide (**13b**)

White solid; yield 78%, 0.4g, m.p. 202-204 °C; IR (KBr, cm^{-1}) 3443, 3035, 1655, 1295, 1130. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.08 (d, J = 1.8 Hz, 1H), 8.13 – 8.06 (m, 2H), 7.95 –

7.90 (m, 2H), 7.75 (d, J = 2.0 Hz, 1H), 7.77 (d, J = 2.4 Hz, 2H), 7.55 – 7.47 (m, 2H), 7.43 – 7.37 (m, 2H), 7.23 –7.28 (m, 2H), 7.15 – 7.14 (m, 1H), 7.12 (d, J = 0.8 Hz, 1H), 6.91 (d, J = 2.1 Hz, 2H), 5.01 (s, 1H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H).^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.80, 168.26, 154.83, 142.73, 138.52, 133.81, 129.31, 129.01, 128.21, 126.22, 125.21, 124.80, 121.81, 121.64, 121.01, 119.86, 83.87, 61.65, 53.34, 49.68. LCMS(m/z): calcd. for $C_{32}H_{30}N_4OS$ 518.22, found 519.23 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(4-chlorophenyl)piperazin-1-yl)acetamide (**13c**)
Brown solid; yield 92%, 0.42g, m.p. 203-204 °C; IR (KBr, cm^{-1}) 3465, 3046, 1645, 1292, 1135, 755. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.09 (d, J = 1.8 Hz, 1H), 8.12 – 8.06 (m, 2H), 7.75 (d, J = 2.0 Hz, 1H), 7.71 (d, J = 2.0 Hz, 1H), 7.54 – 7.48 (m, 2H), 7.43 – 7.37 (m, 1H), 7.15 – 7.14 (m, 1H), 7.14 (d, J = 0.8 Hz, 1H), 6.73 (d, J = 2.3 Hz, 2H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.85, 168.36, 154.95, 149.21, 138.22, 134.42, 129.60, 120.99, 126.37, 125.17, 124.90, 121.88, 121.44, 121.11, 119.77, 114.46, 61.16, 51.37, 49.56. LCMS (m/z): calcd. for $C_{25}H_{23}ClN_4OS$ 462.13, found 463.14 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(2-fluorophenyl)piperazin-1-yl)acetamide (**13d**)
Brown solid; yield 87%, 0.38g, m.p. 203-204 °C; IR (KBr, cm^{-1}) 3470, 3045, 1640, 1290, 1135, 1065. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.13 (d, J = 1.9 Hz, 1H), 8.12 – 8.07 (m, 2H), 7.91 – 7.88 (m, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.15 – 7.13 (m, 1H), 6.91 – 7.06 (m, 3H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.65, 168.46, 155.75, 154.67, 138.51, 137.22, 133.82, 129.09, 126.54, 125.57, 125.27, 124.48, 122.87, 121.88, 121.64, 119.81, 116.46, 115.56, 63.16, 54.37, 51.56. LCMS (m/z): calcd. for $C_{25}H_{23}FN_4OS$ 446.15, found 447.14 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(pyridin-2-yl)piperazin-1-yl)acetamide (**13e**)
Brown solid; yield 82%, 0.34g, m.p. 208-209 °C; IR (KBr, cm^{-1}) 3435, 3043, 1660, 1290, 1130. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (s, 1H), 8.11 (d, J = 1.9 Hz, 1H), 8.10 – 8.06 (m, 2H), 8.04 (d, J = 2.0 Hz, 1H), 7.94 – 7.90 (m, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.55 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.15 – 7.13 (m, 1H), 6.70 (d, J = 2.1 Hz, 1H), 6.62 – 629 (m,

1H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.85, 168.56, 158.21, 154.93, 148.25, 138.49, 138.32, 133.83, 129.01, 126.65, 125.52, 124.82, 121.98, 121.64, 119.89, 118.12, 106.67, 63.65, 51.34, 47.58. LCMS(m/z): calcd. for $C_{24}H_{23}N_5OS$ 429.16, found 430.17 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(p-tolyl)piperazin-1-yl)acetamide (**13f**)

Brown solid; yield 65%, 0.28g, m.p. 210-211 °C; IR (KBr, cm^{-1}) 3465, 3050, 1665, 1290, 1130. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (s, 1H), 8.11 (d, J = 1.9 Hz, 1H), 8.10 – 8.06 (m, 2H), 7.94 – 7.90 (m, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.15 – 7.13 (m, 1H), 7.12 (d, J = 0.8 Hz, 1H), 6.91 (d, J = 2.1 Hz, 1H), 6.89 (d, J = 2.1 Hz, 1H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.42, 167.46, 154.15, 148.80, 140.01, 134.94, 129.84, 129.76, 129.44, 128.51, 126.36, 125.10, 123.02, 121.61, 119.45, 116.62, 62.02, 53.60, 50.04, 20.49. LCMS (m/z): calcd. for $C_{26}H_{26}N_4OS$ 442.18, found 443.19 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(4-hydroxyphenyl)piperazin-1-yl)acetamide (**13g**)

Brown solid; yield 94%, 0.41g, m.p. 232-234 °C; IR (KBr, cm^{-1}) 3575, 3430, 3040, 1640, 1290, 1135. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.14 (d, J = 1.7 Hz, 1H), 8.09 – 8.04 (m, 2H), 7.97 – 7.90 (m, 1H), 7.78 (d, J = 2.2 Hz, 1H), 7.72 (d, J = 2.1 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.15 – 7.13 (m, 1H), 7.12 (d, J = 0.8 Hz, 1H), 6.79 (d, J = 2.2 Hz, 1H), 6.59 (d, J = 2.2 Hz, 1H), 5.95 (s, 1H) 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.78, 168.46, 154.49, 148.21, 148.03, 138.52, 133.82, 129.02, 126.59, 125.30, 124.51, 121.88, 121.65, 119.87, 116.96, 115.78, 61.16, 51.37, 48.46. LCMS (m/z): calcd. for $C_{25}H_{24}N_4O_2S$ 444.16, found 445.18 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetamide (**13h**)

Yellow solid; yield 94%, 0.44g, m.p. 246-247 °C; IR (KBr, cm^{-1}) 3435, 3030, 1650, 1530, 1320, 1295, 1130. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.14 (d, J = 2.0 Hz, 1H), 8.09 (d, J = 2.1 Hz, 2H), 8.12 – 8.06 (m, 2H), 7.75 (d, J = 2.0 Hz, 1H), 7.79 (d, J = 2.0 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.15 – 7.14 (m, 1H), 7.04 (d, J = 0.8 Hz, 2H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.86, 168.36, 155.87,

154.55, 138.52, 137.49, 134.42, 129.09, 126.37, 125.17, 124.80, 124.67, 121.88, 121.64, 119.77, 112.46, 61.16, 51.37, 49.36. LCMS (m/z): calcd. for $C_{25}H_{23}N_5O_3S$ 473.15, found 474.15 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(2-chlorophenyl)piperazin-1-yl)acetamide (**13i**)

Green solid; yield 63%, 0.28g, m.p. 199-200 °C; IR (KBr, cm^{-1}) 3465, 3040, 1640, 1287, 1146, 745. ^1H NMR (400 MHz, $CDCl_3$) δ 9.37 (s, 1H), 8.06 (d, J = 1.8 Hz, 1H), 8.10 – 8.04 (m, 2H), 7.88 (d, J = 2.0 Hz, 1H), 7.71 (d, J = 2.0 Hz, 2H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.15 – 7.14 (m, 1H), 7.14 (d, J = 0.8 Hz, 1H), 6.70 –6.73 (m, 1H), 6.71 (d, J = 2.1 Hz, 1H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H) ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.55, 168.56, 154.67, 150.10, 138.50, 133.80, 130.09, 129.09, 129.01, 127.74, 126.66, 125.37, 124.98, 124.58, 123.77, 121.88, 121.64, 119.81, 63.26, 54.37, 51.46. LCMS (m/z): calcd. for $C_{25}H_{23}ClN_4OS$ 462.12, found 463.13 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(3-hydroxyphenyl)piperazin-1-yl)acetamide (**13j**)

Brown solid; yield 81%, 0.35g, m.p. 228-229 °C; IR (KBr, cm^{-1}) 3568, 3438, 3013, 1645, 1292, 1135. ^1H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.14 (d, J = 1.7 Hz, 1H), 8.09 – 8.04 (m, 2H), 7.97 – 7.90 (m, 1H), 7.78 (d, J = 2.2 Hz, 1H), 7.72 (d, J = 2.1 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.15 – 7.13 (m, 1H), 6.59 (d, J = 2.2 Hz, 1H), 6.33 (d, J = 2.2 Hz, 1H), 6.21(s, 1H), 5.95 (s, 1H) 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.65, 168.66, 159.47, 154.50, 151.32, 138.50, 133.81, 131.23, 129.02, 126.76, 125.36, 124.68, 121.80, 121.66 119.88, 108.55, 107.01, 100.10, 63.26, 54.37, 51.46. LCMS (m/z): calcd. for $C_{25}H_{24}N_4O_2S$ 444.16, found 445.17 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)acetamide (**13k**)

Pale green solid; yield 91%, 0.5g, m.p. 199-200 °C; IR (KBr, cm^{-1}) 3438, 3064, 1655, 1288, 1135, 1085. ^1H NMR (400 MHz, $CDCl_3$) δ 9.37 (s, 1H), 8.08 (d, J = 1.8 Hz, 1H), 8.13 – 8.06 (m, 2H), 7.75 (d, J = 2.0 Hz, 1H), 7.77 (d, J = 2.4 Hz, 1H), 7.55 – 7.47 (m, 1H), 7.23 –7.28 (m, 2H), 7.15 – 7.14 (m, 4H), 7.12 – 7.09 (m, 4H), 5.01 (s, 1H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.81, 168.36, 160.19, 154.43, 138.52,

138.31, 129.81, 129.01, 126.60, 126.22, 125.31, 124.50, 121.81, 121.64, 119.89, 116.09, 84.87, 62.65, 53.34, 49.68. LCMS(m/z): calcd. for Chemical Formula: $C_{32}H_{28}F_2N_4OS$ 554.19, found 555.20 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(3-methoxyphenyl)piperazin-1-yl)acetamide (**13l**)

Pale green solid; yield 69%, 0.31g, m.p. 195-196 °C; IR (KBr, cm^{-1}) 3467, 3065, 1665, 1285, 1210, 1130. ^1H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 8.13 (d, J = 1.9 Hz, 1H), 8.10 – 8.06 (m, 2H), 7.94 – 7.90 (m, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.15 – 7.13 (m, 1H), 6.53 (d, J = 2.1 Hz, 1H), 6.39 (d, J = 2.1 Hz, 1H), 6.34 (s, 1H), 3.69 (s, 3H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.65, 168.65, 161.48, 154.50, 150.32, 138.51, 133.80, 130.61, 129.02, 126.76, 125.36, 124.68, 121.80, 121.65 119.88, 110.55, 106.61, 98.10, 63.26, 55.01, 54.37, 49.86. LCMS (m/z): calcd. for $C_{26}H_{26}N_4O_2S$ 458.18, found 459.19 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(1-phenylethyl)piperazin-1-yl)acetamide (**13m**)

Pale green solid; yield 79%, 0.35g, m.p. 127-127 °C; IR (KBr, cm^{-1}) 3460, 3045, 1645, 1292, 1135. ^1H NMR (400 MHz, $CDCl_3$) δ 9.44 (s, 1H), 8.10 (d, J = 1.9 Hz, 1H), 8.12 – 8.08 (m, 2H), 7.95 – 7.91 (m, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.71 (d, J = 2.0 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.40 – 7.32 (m, 1H), 7.25 – 7.29 (m, 2H), 7.26 (d, J = 1.2 Hz, 2H), 4.19 – 4.15 (m, 1H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H), 1.12 (d, J = 2.3 Hz, 3H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.81, 168.36, 154.43, 145.67, 138.52, 138.31, 129.81, 129.01, 128.82, 128.54, 127.32, 126.61, 125.31, 124.51, 121.81, 121.64, 73.17, 62.65, 53.34, 49.68, 20.02. LCMS(m/z): calcd. for Chemical Formula: $C_{27}H_{28}N_4OS$ 456.19, found 457.21 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-benzylpiperazin-1-yl)acetamide (**13n**)

Pale green solid; yield 85%, 0.37g, m.p. 180-182 °C; IR (KBr, cm^{-1}) 3454, 3056, 1650, 1290, 1137. ^1H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 8.10 (d, J = 1.9 Hz, 1H), 8.12 – 8.06 (m, 2H), 7.95 – 7.90 (m, 1H), 7.76 (d, J = 2.1 Hz, 1H), 7.71 (d, J = 2.0 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 7.36 – 7.32 (m, 2H), 7.26 – 7.29 (m, 1H), 7.24 (d, J = 1.3 Hz, 2H), 3.79 (s, 2H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.83, 168.26, 154.53, 138.71, 138.52, 133.82, 129.01, 128.87, 128.45, 127.32, 126.32, 125.31,

124.52, 121.98, 121.64, 119.86, 65.54, 61.15, 51.34, 49.58. LCMS(m/z): calcd. for $C_{26}H_{26}N_4OS$ 442.18, found 443.20 $[M + H]^+$.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(pyrimidin-2-yl)piperazin-1-yl)acetamide (**13o**)

Brown solid; yield 76%, 0.32g, m.p. 167-168 °C; IR (KBr, cm^{-1}) 3460, 3045, 1640, 1290, 1130. ^1H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.47 (d, J = 2.0 Hz, 2H), 8.11 (d, J = 1.9 Hz, 1H), 8.10 – 8.06 (m, 2H), 8.04 (d, J = 2.0 Hz, 1H), 7.94 – 7.90 (m, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.43 – 7.37 (m, 1H), 6.90 – 6.93 (m, 1H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.85, 168.56, 158.21, 154.93, 148.25, 138.49, 138.32, 133.83, 129.01, 126.65, 124.82, 121.98, 121.64, 119.89, 118.12, 106.67, 63.65, 51.34, 47.58. LCMS(m/z): calcd. for $C_{23}H_{22}N_6OS$ 430.16, found 431.17 $[M + H]^+$.

tert-butyl4-(2-((4-(benzo[d]thiazol-2-yl)phenyl)amino)-2-oxoethyl)-3-oxopiperazine-1-carboxylate (**13p**)

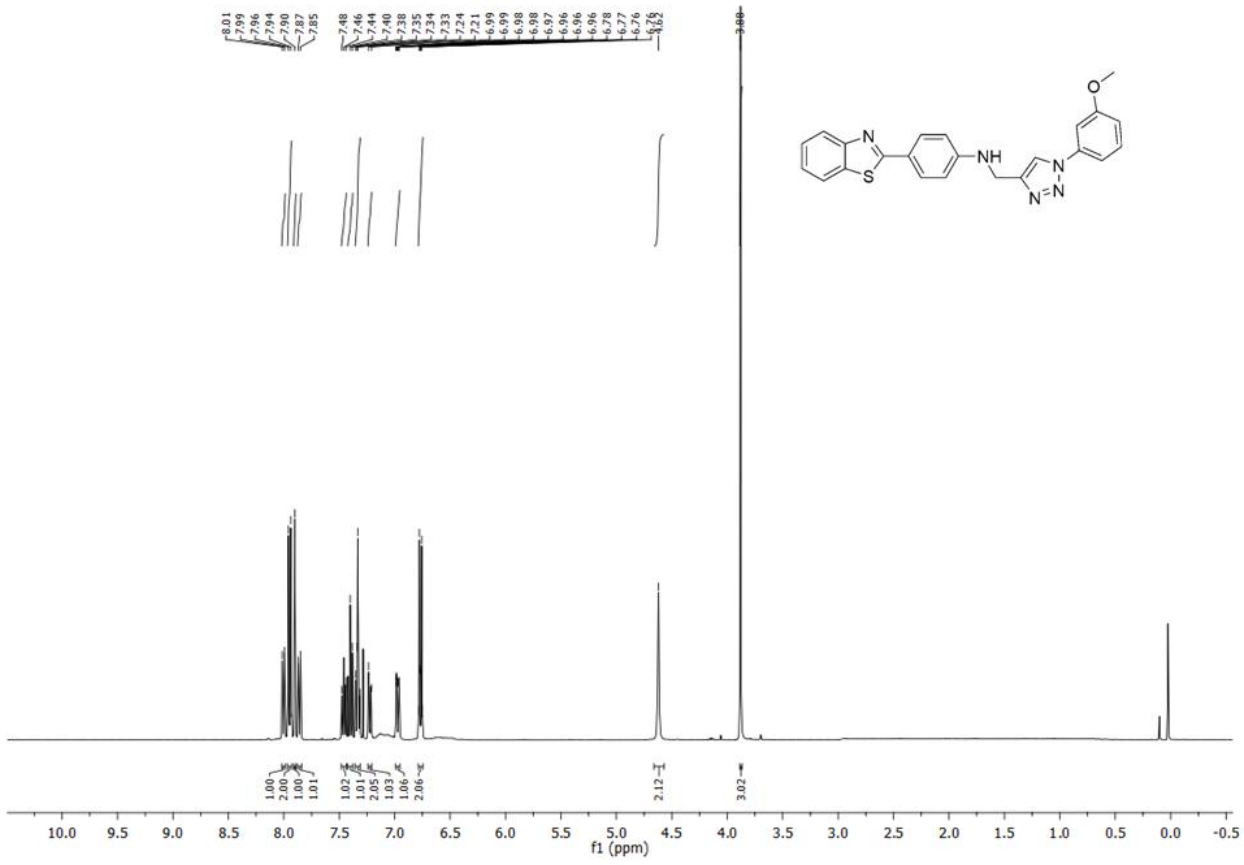
Brown solid; yield 87%, 0.4g, m.p. 150-151 °C; IR (KBr, cm^{-1}) 3468, 3042, 1735, 1643, 1293, 1220, 1132. ^1H NMR (400 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.10 (d, J = 1.9 Hz, 1H), 8.12 – 8.06 (m, 2H), 7.76 (d, J = 2.1 Hz, 1H), 7.71 (d, J = 2.0 Hz, 2H), 7.54 – 7.48 (m, 1H), 7.43 – 7.37 (m, 1H), 3.89 (s, 2H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 1.28 (s, 9H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.83, 168.26, 163.21, 154.53, 138.71, 138.52, 133.82, 129.01, 128.87, 128.45, 124.52, 79.08, 65.54, 61.15, 53.21, 51.34, 50.12, 49.48, 32.58, 28.12. LCMS(m/z): calcd. for $C_{24}H_{26}N_4O_4S$ 466.17, found 467.18 $[M + H]^+$

tert-butyl4-(2-((4-(benzo[d]thiazol-2-yl)phenyl)amino)-2-oxoethyl)piperazine-1-carboxylate (**13q**)

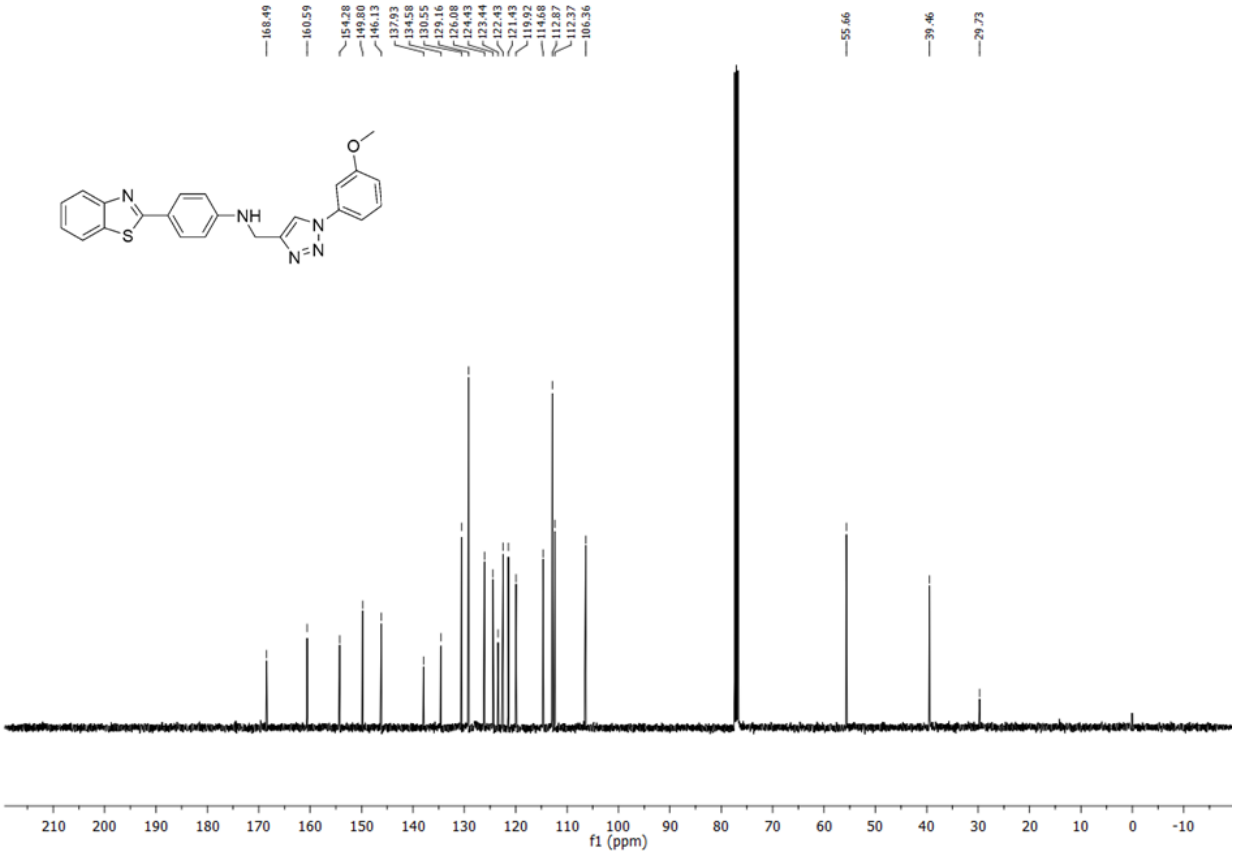
Pale green solid; yield 78%, 0.35g, m.p. 226-227 °C; IR (KBr, cm^{-1}) 3465, 3046, 1740, 1645, 1292, 1215, 1135. ^1H NMR (400 MHz, $CDCl_3$) δ 9.37 (s, 1H), 8.09 (d, J = 1.8 Hz, 1H), 8.12 – 8.07 (m, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.71 (d, J = 2.0 Hz, 1H), 3.27 (d, J = 2.9 Hz, 4H), 3.25 (s, 2H), 2.86 – 2.82 (m, 4H), 1.28 (s, 9H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 169.83, 168.26, 154.93, 154.17, 138.12, 133.62, 129.61, 129.01, 126.32, 124.82, 121.98, 79.18, 61.15, 53.81, 51.34, 49.58, 45.32, 28.12. LCMS(m/z): calcd. for $C_{24}H_{28}N_4O_3S$ 452.19, found 453.20 $[M + H]^+$.

General procedure for the synthesis of 4-(benzo[d]thiazol-2-yl)-N-((substituted phenyl-1H-1,2,3-triazol-4-yl)methyl)aniline (**15a-k**)

Alkylation of 2-(4-aminophenyl)benzothiazole (**11)** with propargyl bromide in *N,N*-dimethylformamide yielded compound **14** in 80% after column purification with pet ether and ethyl acetate (20%). To a stirred solution of compound **14** (0.3g, 1.1363 mmol) and substituted azide (1.25 mmol) in *tert*-butanol and water (1:1) (3 mL), $CuSO_4.5H_2O$ (56.74mg, 0.2272 mmol) and sodium ascorbate (45.01mg, 0.2272 mmol) were added and the reaction mixture was stirred at RT for 2 h. After completion of the reaction, as indicated by TLC, *tert*-butanol was removed under reduced pressure. The residue was extracted with ethyl acetate (3 x10 mL) and combined organic layers were collected and washed with saturated brine solution, dried over anhydrous $MgSO_4$ and concentrated in vacuo to get the crude product. The product was further purified by column chromatography using 20-30% ethyl acetate in pet ether to afford the title compounds.



^{1}H NMR spectrum of compound **15b**



^{13}C NMR spectrum of compound **15b**

4-(benzo[d]thiazol-2-yl)-N-((1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15a**)

Brown solid; yield 91%, 0.46g, m.p. 213-214 °C; IR (KBr, cm^{-1}) 3465, 3046, 1292, 1035. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (dd, J = 8.1, 1.2 Hz, 1H), 7.98 – 7.91 (m, 2H), 7.91 (d, J = 0.8 Hz, 1H), 7.85 (dd, J = 8.0, 1.3 Hz, 1H), 7.51 (d, J = 1.9 Hz, 2H), 7.39 – 7.36 (m, 2H), 7.23 (dd, J = 8.0, 2.1 Hz, 1H), 6.97 (dd, J = 8.3, 2.5 Hz, 1H), 6.88 – 6.85 (m, 2H), 4.62 (s, 2H). ^{13}C NMR (101 MHz, CDCl3) δ 168.49, 160.59, 154.29, 149.80, 146.14, 137.23, 134.48, 131.21, 130.55, 129.67, 126.08, 124.43, 121.64, 120.12, 119.92, 114.68, 112.87, 112.38, 106.36, 55.66, 39.47. LCMS(m/z): calcd. for $C_{23}H_{16}F_3N_5S$ 451.11, found 452.19 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15b**)

Brown solid; yield 67%, 0.31g, m.p. 152-154 °C; IR (KBr, cm^{-1}) 3465, 3046, 1292, 1210. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, J = 8.1, 1.2 Hz, 1H), 7.97 – 7.93 (m, 2H), 7.90 (d, J = 0.8

Hz, 1H), 7.86 (dd, J = 8.0, 1.3 Hz, 1H), 7.49 – 7.38 (m, 2H), 7.36 – 7.30 (m, 2H), 7.22 (dd, J = 8.0, 2.1 Hz, 1H), 6.97 (dd, J = 8.3, 2.5 Hz, 1H), 6.78 – 6.75 (m, 2H), 4.62 (s, 2H), 3.88 (s, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 168.49, 160.59, 154.29, 149.80, 146.14, 137.93, 134.58, 130.55, 129.17, 126.08, 124.43, 123.45, 121.44, 119.92, 114.68, 112.87, 112.38, 106.36, 55.66, 39.47, 29.73. LCMS(m/z): calcd. for $C_{23}H_{19}N_5OS$ 413.13, found 414.15 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(2-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15c**)
Brown solid; yield 87%, 0.45g, m.p. 161-162 °C; IR (KBr, cm^{-1}) 3460, 3040, 1290, 575. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, J = 8.1, 1.2 Hz, 1H), 7.96 – 7.94 (m, 2H), 7.90 (d, J = 0.8 Hz, 1H), 7.86 (dd, J = 8.0, 1.3 Hz, 1H), 7.49 – 7.38 (m, 2H), 7.36 – 7.30 (m, 2H), 7.22 (dd, J = 8.0, 2.1 Hz, 1H), 6.97 (dd, J = 8.3, 2.5 Hz, 1H), 6.78 – 6.75 (m, 2H), 4.62 (s, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 168.53, 154.29, 149.82, 145.14, 136.44, 134.58, 133.94, 131.30, 129.15, 128.55, 126.10, 124.44, 123.87, 123.46 , 122.45, 121.44, 118.61, 112.97, 114.12, 39.48. LCMS (m/z): calcd. for $C_{22}H_{16}BrN_5S$ 461.03, found 462.11 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15d**)
Brown solid; yield 81%, 0.38g, m.p. 223-225 °C; IR (KBr, cm^{-1}) 3455, 3068, 1285, 755. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.97 (m, 2H), 7.95 (d, J = 2.0 Hz, 2H), 7.93 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.0, 1.2 Hz, 1H), 7.81 (s, 1H), 7.53 – 7.47 (m, 1H), 7.49 (d, J = 1.9 Hz, 2H), 6.79 (d, J = 2.0 Hz, 2H), 6.77 (d, J = 2.0 Hz, 1H), 4.67 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.52, 154.26, 149.73, 140.24, 139.91, 134.37, 131.59, 129.28, 129.14, 128.84, 126.09, 125.12, 124.43, 123.75, 122.04, 119.14 114.01, 39.51. LCMS (m/z): calcd. for $C_{22}H_{16}ClN_5S$ 417.09, found 418.10 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15e**)
Brown solid; yield 72%, 0.33g, m.p. 208-209 °C; IR (KBr, cm^{-1}) 3472, 3058, 1298. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.99 (m, 2H), 7.95 (d, J = 2.0 Hz, 2H), 7.93 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.0, 1.2 Hz, 1H), 7.89 (s, 1H), 7.53 – 7.47 (m, 1H), 7.39 (d, J = 1.9 Hz, 2H), 6.81 (d, J = 2.1 Hz, 2H), 6.78 (d, J = 2.1 Hz, 1H), 4.66 (s, 2H), 2.05 (m, 2H), 1.32 (t, J = 5.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.52, 154.26, 149.73, 144.56, 140.24, 139.91, 134.87, 134.37,

131.59, 129.28, 129.14, 128.84, 125.12, 124.43, 123.75, 119.14, 114.01, 39.51, 29.05, 14.29. LCMS (m/z): calcd. for $C_{24}H_{21}N_5S$ 411.15, found 412.16 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(2-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15f**)
Brown solid; yield 86%, 0.49g, m.p. 155-157 °C; IR (KBr, cm^{-1}) 3469, 3043, 1298, 525. ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.97 (m, 2H), 7.95 (d, J = 2.0 Hz, 1H), 7.93 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.0, 1.2 Hz, 1H), 7.79 (s, 1H), 7.53 – 7.47 (m, 1H), 7.44 (dd, J = 7.9, 5.1 Hz, 2H), 7.33 (dd, J = 8.2, 7.3 Hz, 1H), 7.27 – 7.20 (m, 1H), 6.79 (d, J = 2.0 Hz, 1H), 6.77 (d, J = 2.0 Hz, 1H), 4.67 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.51, 154.28, 149.83, 145.29, 140.24, 139.91, 134.57, 131.59, 129.28, 129.14, 127.84, 126.09, 124.43, 123.75, 123.43, 122.43, 121.44, 119.14 114.01, 39.50. LCMS (m/z): calcd. for $C_{22}H_{16}IN_5S$ 509.07, found 510.09 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15g**)
Brown solid; yield 75%, 0.36g, m.p. 193-194 °C; IR (KBr, cm^{-1}) 3439, 3065, 1545, 1336, 1292. ^1H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 2.1 Hz, 2H), 8.05 – 7.99 (m, 3H), 7.94 (d, J = 2.1 Hz, 2H), 7.93 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.0, 1.2 Hz, 1H), 7.81 (s, 1H), 7.53 – 7.47 (m, 1H), 6.79 (d, J = 2.0 Hz, 2H), 4.67 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.52, 154.26, 149.73,147.90, 142.24, 139.91, 134.37, 129.28, 129.14, 128.84, 125.12, 124.43, 123.95, 122.04, 120.14, 119.14 114.01, 39.47.LCMS (m/z): calcd. for $C_{22}H_{16}N_6O_2S$ 428.11, found 429.18 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(2,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15h**)
Brown solid; yield 87%, 0.44g, m.p. 203-204 °C; IR (KBr, cm^{-1}) 3460, 3040, 1290, 785. ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.97 (m, 2H), 7.95 (d, J = 2.0 Hz, 2H), 7.93 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.0, 1.2 Hz, 1H), 7.81 (s, 1H), 7.69 (s, 1H), 7.53 – 7.47 (m, 1H), 7.51 (d, J = 1.9 Hz, 1H), 7.38 (d, J = 1.9 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 4.70 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.52, 154.26, 149.73, 144.64, 139.91, 135.57, 132.78, 132.59, 130.12, 130.04, 129.67, 129.28, 129.14, 128.84, 126.69, 125.32, 124.53, 119.14, 114.01, 39.51. LCMS (m/z): calcd. for $C_{22}H_{15}Cl_2N_5S$ 451.04, found 452.09 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15i**)

Brown solid; yield 73%, 0.36g, m.p. 212-214 °C; IR (KBr, cm^{-1}) 3460, 3040, 1290, 1075, 755. ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.96 (m, 2H), 7.95 (d, J = 2.0 Hz, 2H), 7.93 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.0, 1.2 Hz, 1H), 7.81 (s, 1H),7.79 (d, J = 2.0 Hz, 1H), 7.49 – 7.47 (m, 1H), 7.19 – 7.15 (m, 1H), 6.89 (d, J = 2.0 Hz, 2H), 4.67 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.52, 158.67, 154.26, 149.73, 139.91, 133.78, 132.89, 130.12, 129.67, 129.28, 129.14, 128.84, 127.21, 125.32, 124.53, 120.08, 119.14, 116.34, 114.01, 39.50. LCMS (m/z): calcd. for $C_{22}H_{15}ClFN_5S$ 435.07, found 436.11 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15j**)

Brown solid; yield 78%, 0.36g, m.p. 208-210 °C; IR (KBr, cm^{-1}) 3460, 3049, 1298, 756. ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (dd, J = 8.1, 1.2 Hz, 1H), 7.98 – 7.91 (m, 2H), 7.90 (d, J = 0.8 Hz, 1H), 7.86 (dd, J = 8.0, 1.3 Hz, 1H), 7.51 (d, J = 1.9 Hz, 2H), 7.39 – 7.36 (m, 2H), 7.22 (dd, J = 8.0, 2.1 Hz, 1H), 6.97 (dd, J = 8.3, 2.5, Hz, 1H), 6.88 – 6.85 (m, 2H), 4.62 (s, 2H). ^{13}C NMR (101 MHz, CDCl3) δ 168.49, 160.59, 154.29, 149.80, 146.14, 137.23, 134.48, 131.21, 130.55, 129.67, 126.08, 124.43, 121.64, 119.92, 114.68, 112.87, 112.38, 106.36, 55.66, 39.47. LCMS(m/z): calcd. for $C_{22}H_{16}ClN_5S$ 417.08, found 418.09 $[M + H]^+$.

4-(benzo[d]thiazol-2-yl)-N-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)aniline (**15k**)

Brown solid; yield 83%, 0.37g, m.p. 213-214 °C; IR (KBr, cm^{-1}) 3460, 3075, 1287, 1065. ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.99 (m, 2H), 7.96 (d, J = 2.0 Hz, 1H), 7.93 (d, J = 2.0 Hz, 1H), 7.85 (dd, J = 8.0, 1.2 Hz, 1H), 7.81 (s, 1H), 7.60 – 7.57 (m, 1H), 7.49 (d, J = 2.0 Hz, 2H), 7.29 – 7.25 (m, 2H), 6.79 (d, J = 2.0 Hz, 2H), 4.60 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.52, 162.96, 154.26, 149.73, 140.24, 139.91, 134.37, 132.49, 129.68, 126.09, 125.12, 124.43, 123.75, 122.54, 119.14, 115.23, 114.01, 39.59. LCMS (m/z): calcd. for $C_{22}H_{16}FN_5S$ 401.11, found 402.17 $[M + H]^+$.

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Chapter V

Synthesis of pyrrolo[2,3-*b*]pyridine analogues as antiproliferative agents

Chapter 5

Synthesis of pyrrolo[2,3-*b*]pyridine analogues as antiproliferative agents

5.1. Introduction

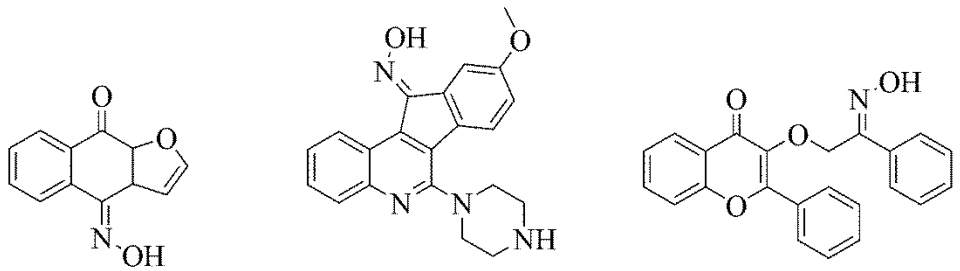
A systematic review of current anticancer literature of a variety of small heterocyclic scaffolds, such as nitrogen heterocycles, reveals an advantage of antiproliferative agents as they mimic numerous biomolecules. Among them fused heterocycles such as 1*H*-pyrrolo[2,3-*b*]pyridine (7-azaindole) is significant scaffold in medicinal chemistry. 1*H*-pyrrolo[2,3-*b*]pyridines are found in numerous natural products such as variolins, isolated from antarctic sponge *Kirk-patrickia varialosa* [1]. 7-azaindoles are biologically competent organic compounds with dissimilar type of actions, such as anti-proliferative [2], protein-kinase inhibition [3], anti-inflammatory [4], antiviral [5], influenza PB2 inhibition [6], inhibition of mixed lineage kinase 3 (MLK3) [7] and selective KIT tyrosine kinase Inhibition [8].

Many substituent modifications happened at different positions of 7-azindole and their biological activities were evaluated. By changing the substitutions at 3rd and 5th positions of 7-azaindole, [3,5-*d*]-7-azaindole analogues through fragment-based growing strategy [9] as phosphatidylinositol-3-kinase alpha (PI3Kα) inhibitors were developed and they also exhibited antiangiogenic effect on cancer cells [10]. 7-azaindole containing 4-pyridyl group at the C-3 position, sulfonamide group at C-5 position analog influenced tropomyosin-related kinase A (Trk A) binding affinity (1.67nM) and exhibited good antiproliferative activity against MCF7 cell line. This analog exhibited strong apoptotic and antiangiogenic effects by inhibiting HIF-1α and vascular endothelial growth factor (VEGF) expression and repressed the angiogenic process by inhibiting endothelial cell migration and tube formation [11]. 7-azaindole containing rebeccamycin analogues have strong antiproliferative activity they are, less toxic and arrest the cell cycle in G_2/M phase at 0.25 μM than rebeccamycin [12]. Cytotoxicity against various cancer cell lines is considerably enhanced by replacing the amine ligands of cisplatin by 7-azaindole analogues [13, 14]. The 7-azaindole ring scaffold is capable of playing a main role in controlling the chemical and biological properties like cellular distribution, cellular accumulation, dissimilar

effects at the stage of cell cycle regulation, reduced propensity for DNA adduct repair and binding to DNA [15].

7-azaindole–chloro pyridine analogues inhibit the Cdc7 (cell division cycle 7) kinase which is necessary for activating the DNA replicative complex at the beginning of replication [16-20]. (*Z*)-2-(benzylamino)-5-(1*H*-pyrrolo[2,3-*b*]pyridin-3-ylmethylene)-1,3-thiazol-4(5H)-one showed 7nM IC_{50} value and it acts as a potent ATP mimetic inhibitor of Cdc7 kinase [21]. 7-azaindole core with 6-methyl substitution showed potent *in vitro* anticancer activity with enhanced metabolic stability, solubility, and oral bioavailability [22]. 6-substituted pyrrolo[2,3-*b*]pyridine-1-carboxamide analogues are new class of PARP-1 [Poly(ADP-ribose) polymerase] inhibitors and are involved in maintaining DNA integrity and in regulation of programmed cell death [23, 24] and showed potent *in vitro* and *in vivo* activity when used at a lower dose [25]. 2,5 disubstituted 7-azaindole analog, methyl 5-(2-chloro-6-methylbenzylamino)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate potently inhibited Abl and Src kinases with IC_{50} values 1.4 nM and 3.4 nM respectively [26]. Hence, substituted 7-azaindoles can be explored for the synthesis of novel anticancer agents.

It is very interesting to know that several pharmacophores like naphthofuranones, methoxyphenyl oximes, and piperazinyl indenoquinolinone having oxime (hydroxylamino) as a functional group play an important role as anticancer compound. Tseng C.H. *et. al.*, synthesized (*Z*)-4-(hydroxyimino)naphtho[2,3-*b*]furan-9(4H)-one derivative which exhibited potent antiproliferative activity against selected cell lines. Oxime containing anticancer compound is depicted in **Figure 5.1** [27-29].



$GI_{50} = 0.82 \pm 0.02$ μM $GI_{50} = 1.74 \pm 0.47$ μM $GI_{50} = 1.8 \pm 0.20$ μM

J **K** **L**

Figure 5.1: Oxime containing anticancer compounds [27-29].

1,2,3-triazoles are imperative class of heterocycles and play a major role in medicinal chemistry with antifungal [30, 31], antibacterial [32, 33], antiallergic [34], anti-inflammatory [35] and anticancer activities [36]. In recent years, copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction has become a synthetic cornerstone for conjugating building blocks with diverse functionalities [37].

Singh *et. al.*, reported 1,2,3-triazole tethered β-lactam-chalcone bifunctional hybrids as anticancer agents [38]; Duan *et. al.*, synthesized 1,2,3-triazole-dithiocarbamate hybrids [39] as well as 1,2,3-triazole-dithiocarbamate-urea hybrids [40] and evaluated them for the anticancer activity against selected human tumor cell lines.

Some of the compounds exhibited excellent broad spectrum anticancer activity. Ahmed *et. al.*, synthesized flavone-triazole-tetrahydropyran conjugates and evaluated the compounds for anticancer activity, in which most of the compounds exhibited IC_{50} in the range of 0.61-1.68 µM [41]. Ma *et. al.*, synthesized 1,2,3-triazole-pyrimidine hybrids, which showed IC_{50} values ranging from 1.42 to 6.52 µM against various cancer cell lines [42]. Some of anticancer 1,2,3-triazoles are shown in **Figure 5.2**.

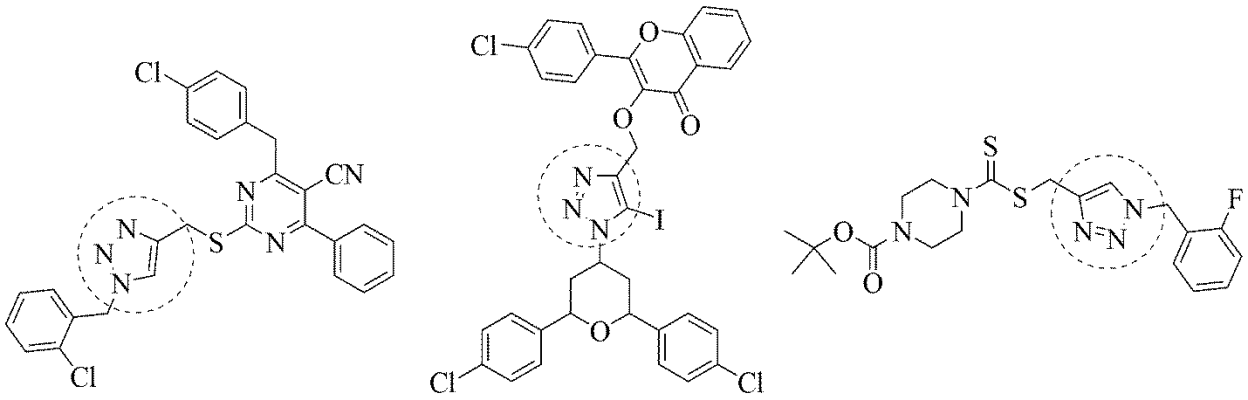


Figure 5.2: Examples of some 1,2,3-triazole based anticancer agents [39,41,42]

Our newly designed scaffold consists of three prime components, i.e., 7-azaindole as a core moiety, oxime at 3rd position of 7-azaindole, and 1,2,3-triazole at 1st and 3rd position. To explore the alterations of this conserved hinge region oxime, we modified this region with 1,2,3-triazole. Here we report the chemical synthesis of new analogues of 1*H*-pyrrolo[2,3-*b*]pyridine, that are based on some of the major chemotherapeutic pharmacophores in the area of cancer. In this study we designed and synthesized novel 7-azaindole analogues by varying substitutions at 1st and 3rd position [**scheme 6** and **scheme 7**] and evaluated antiproliferative activity on three different human cancer cell lines. One such derivative stood out in these screens (Table1) and was selected as lead molecule for CtDNA binding.

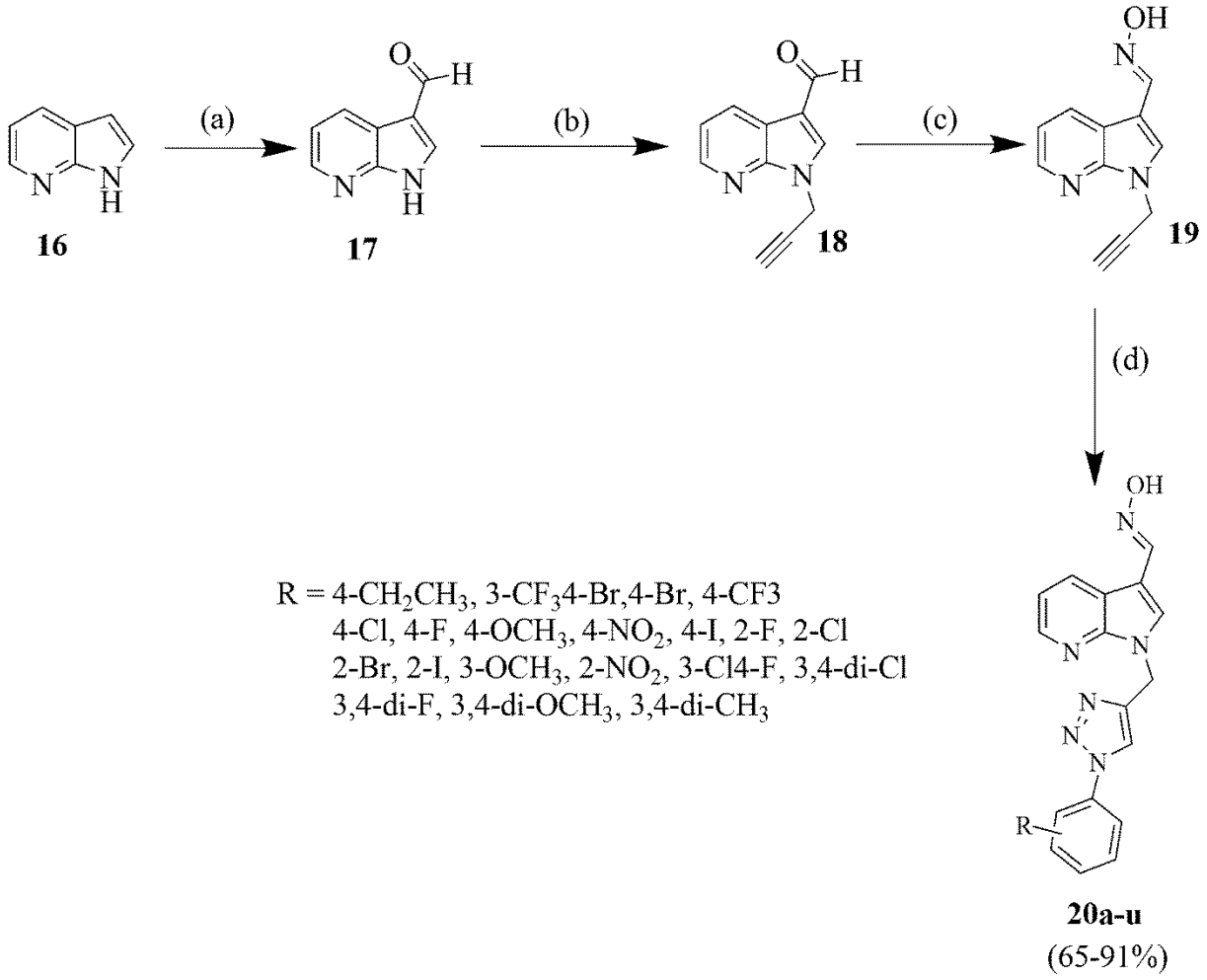
5.2. Results and Discussion

5.2.1. Chemistry

The synthesis of pyrrolo[2,3-*b*]pyridine analogues **20a-u** and **22a-k** described in this study is depicted in **scheme 6** and **scheme 7**. 1*H*-pyrrolo [2,3-*b*]pyridine-3-carbaldehyde **(17)** was prepared using reported procedure of Duff reaction in the presence of hexamethylenetetramine (HMTA), acetic acid (33%) [43].

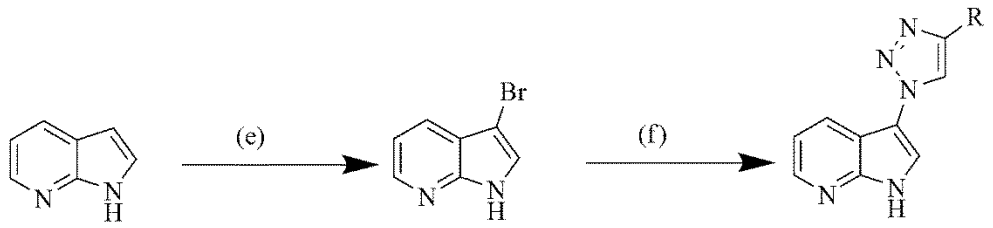
Formylation occurs at C-3 position of 1*H*-pyrrolo [2,3-*b*]pyridine yielding 2 in 75% yield. 1*H*-pyrrolo [2,3-*b*]pyridine-3-carbaldehyde (**17**) was treated with propargyl bromide in the presence of potassium carbonate (K_2CO_3), dimethyl formamide (DMF) to yield 1-(prop-2-ynyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde **(18)**. This alkylation happened at 1st position of 1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde (**17**).

1-(prop-2-ynyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde oxime (**19**) was synthesized by reacting 1-(prop-2-ynyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde (**18**) with hydroxylamine hydrochloride in the presence of ethanol. The title compounds (**20a-u**) were synthesized by click chemistry. 1-(prop-2-ynyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde oxime (**19**) on treatement with various aromatic azides, copper sulfate pentahydrate and sodium ascorbate in aqueous DMF in one pot yielded **20a-u**. The synthetic pathway is shown in **scheme 6**.



Scheme 6: Reagents and conditions: (a) HMTA, $CH_3COOH:H_2O$, (b) Propargyl bromide, K_2CO_3, DMF, RT (c) Hydroxylamine hydrochloride, Ethanol (d) various aromatic azides, $CuSO_4.5H_2O$, Sodium ascorbate, *t*BuOH:H_2O (1:1), 2h.

3-bromo-1*H*-pyrrolo[2,3-*b*]pyridine **(21)** was synthesized by treating pyrrolo[2,3-*b*]pyridine with *N*-bromo succinimide in presence of DMF at RT. 3-bromo-1*H*-pyrrolo [2, 3-*b*] pyridine **(21)** was treated with various aromatic and aliphatic alkynes in the presence of sodium azide, copper sulfate pentahydrate and sodium ascorbate, L-proline, sodium carbonate in aq DMSO (9:1) to yield products (**22a-k**). Synthetic pathway is shown in **scheme 7**. All the synthesized compounds were characterized and confirmed by ^{1}H NMR, ^{13}C NMR and LC-MS.



R = C_6H_5, 4-$CH_3C_6H_4$, 4-$C(CH_3)_3C_6H_4$,
$CH_3(CH_2)_3$, cyclopropyl, $OH(CH_3)_2C$-,
$OH(CH_2)_2CH_2$-, CH_3CH_2OCO-, OH-CH_2-
4-FC_6H_4-, 4-$OCH_3C_6H_4$-

Scheme 7: Reagents and conditions: (a) N-Bromosuccinimide, DMF, RT (b) various alkynes, NaN_3, L-proline, Na_2CO_3, $CuSO_4.5H_2O$, Sodium Ascorbate, DMSO:H_2O (9:1)

In general ^{1}H NMR of all the title compounds displayed one singlet peak of N-1 proton (**22a-k**) which resonated in the range of 12.07-12.89 ppm. A sharp peak due to the proton of oxime OH group (**20a-u**) resonated in the range 10.73-10.80 ppm. One singlet resonated in the range of 7.18-9.01 due to proton of triazole ring. The oxime CH protons, showed singlet in the range 7.92-7.97. A sharp singlet in the range 5.63-5.68 ppm corresponding to the methylene protons was observed.

5.2.2. Antiproliferative activity

In vitro antiproliferative activity of the synthesized compounds **20a-u** and **22a-k** were evaluated against three types of human cancer cell lines; A549 (Lung cancer), HeLa (Cervical cancer) and MDA-MB-231 (Breast cancer) employing sulforhodamine B (SRB) assay method [44, 45]. For *in vitro* chemo sensitivity of tumor cell lines, numerous quick colorimetric assays are available, while MTT [3-(4,5-dimethylthiazolyl-2)-2,5 diphenyl tetrazolium bromide] assay being the most extensively used, recently the US National Cancer Institute (NCI) recommended use of the sulforhodamine B (SRB) protein stain for *in vitro* chemo sensitivity testing. The SRB assay appeared to be more responsive than MTT assay, with better linearity with cell number and higher reproducibility [46, 47]. The growth inhibition data (expressed as GI_{50}) of synthesized compounds **20a-u** and **22a-k** are shown in **Table 5.1**.

Table 5.1: Antiproliferative activity ($^{a}GI_{50}$ μM) and docking scores of compounds (**20a-u** and **22a-k)**

Entry	R	A549	HeLa	MDA-MB-231	Docking Score
20a	4-CH_3CH_2-	3.94±0.32	1.84±0.02	0.46±0.02	-5.161
20b	3-CF_3,4-Br	0.33±0.01	1.35±0.13	1.3±0.09	-4.412
20c	4-Br	0.7±0.01	0.93±0.02	**0.25±0.03**	-5.216
20d	4-CF_3	**0.12±0.01**	0.79±0.05	0.63±0.02	-5.522
20e	4-Cl	1.12±0.08	0.9±0.01	**0.13±0.01**	-5.104
20f	4-F	0.3±0.02	0.86±0.02	1.22±0.07	------
20g	4-OCH_3	1.82±0.07	0.81±0.01	1.51±0.05	-6.37
20h	4-NO_2	**0.24±0.01**	0.92±0.01	2.5±0.12	-5.207
20i	4-I	0.63±0.02	1.19±0.09	1.6±0.02	-5.628
20j	2-F	1.2±0.09	1.18±0.11	1.2±0.08	-5.072
20k	2-Cl	**0.16±0.04**	0.68±0.02	6.3±0.23	-5.618
20l	2-Br	2.69±0.07	0.39±0.05	3.0±0.01	-6.332
20m	2-I	1.17±0.08	0.76±0.02	**0.13±0.01**	-5.145
20n	3-OCH_3	0.78±0.04	0.79±0.03	**0.23±0.02**	-4.833
20o	2-NO_2	0.95±0.02	1.14±0.03	0.41±0.01	-5.399
20p	3-Cl,4-F	0.83±0.03	0.95±0.02	2.7±0.06	-5.378
20q	3,4-di-Cl	1.17±0.06	1.05±0.09	**0.25±0.02**	-5.043
20r	3,4-di-F	0.95±0.02	1.39±0.1	9.3±0.9	-5.471
20s	3,4-di-OCH_3	0.91±0.01	1.91±0.07	**0.14±0.02**	-4.728
20t	3,4-di-CH_3	0.92±0.01	0.97±0.04	6.98±0.56	-5.476
20u	3,4-methylene dioxy	0.98±0.03	1.0±0.03	1.57±0.08	-5.5
22a	C_6H_5-	0.98±0.02	0.92±0.01	9.84±0.59	-7.429
22b	4-$CH_3C_6H_4$-	0.33±0.01	0.89±0.03	6.22±0.17	-7.414
22c	4-$C(CH_3)_3$-C_6H_4-	0.4±0.01	1.03±0.02	0.48±0.02	-7.185
22d	$CH_3(CH_2)_3$-	0.83±0.02	1.86±0.09	15.3±1.8	-7.971
22e	Cyclopropyl	0.61±0.01	1.32±0.08	4.0±0.3	-7.367

22f	$OH(CH_3)_2C$-	0.82±0.01	0.9±0.01	8.4±0.53	**-8.381**
22g	$OH(CH_2)_2CH_2$-	0.81±0.009	0.95±0.02	0.69±0.04	**-8.18**
22h	CH_3CH_2OCO-	1.3±0.16	0.88±0.05	2.59±0.25	-7.906
22i	OH-CH_2-	1.73±0.07	0.93±0.02	4.69±0.5	**-8.663**
22j	4-F-C_6H_4-	**0.18±0.02**	0.7±0.02	**0.25±0.02**	-7.47
22k	4-OCH_3-C_6H_4-	**0.17±0.01**	0.83±0.03	0.45±0.01	-7.267
Crizotinib					-8.123
Doxorubicin		<0.01	0.09±0.001	<0.01	
Paclitaxel		<0.01	0.023±0.002	<0.01	

[a]GI_{50}: 50% Growth inhibition

From the antiproliferative activity results, it is evident that all the synthesized compounds have comparable antiproliferative activity with GI_{50} values ranging from 0.12-9.84 μM. It is observed that, the majority of the compounds tested displayed significant growth inhibition on A549 and MDA MB 231 cancer cell lines as compared to HeLa cancer cell line. Compounds **20d** and **20k** showed potent anticancer activity against lung cancer cell line (A549) with GI_{50} values 0.12μM and 0.16μM and compounds **20e** and **20m** showed potency against breast cancer cell line with GI_{50} value 0.13 μM as compared with the positive controls. While the positive controls, Dox and paclitaxel demonstrated the GI_{50} in the range of <0.01-0.09 μM and <0.01-0.023 μM respectively. Compound **20r** is potent against breast cancer cell line with GI_{50} value 0.14 μM. Compound **22j** inhibited cell growth significantly in three human cancer cell lines with GI_{50} values 0.18 μM against A549, 0.7 μM against HeLa and 0.25 μM against MDA MB 231. The compounds **22a**, **22b**, **22c**, **22d**, **22e**, **22f, 22g, 22h, 22i** and **22k** had shown comparable values against lung, cervical and breast cancer cell lines with GI_{50} values ranging from 0.17-9.84μM as compared to the standard drugs.

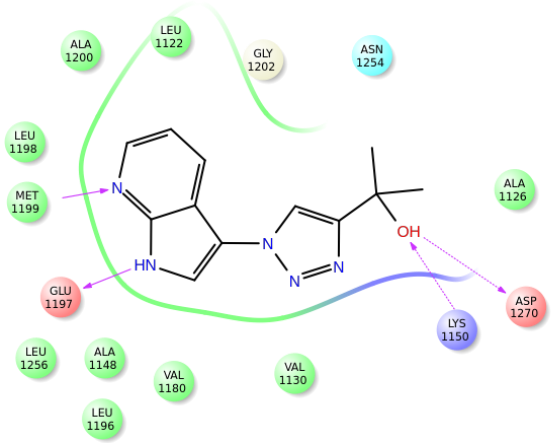
Based on the results of antiproliferative activity, the structure activity relationships (SAR) for these newly synthesized compounds (**20a-u** and **22a-k**) are described as follows. It is interesting to observe that compound with electron withdrawing chloro substitution at ortho-position (**20k**) exhibits good activity (0.16 μM) against A549 cell line compared with other halo substitutions like fluoro (**20j**), bromo (**20l**) and iodo (**20m**). Compound with electron

withdrawing nitro group at ortho position (**20o**) the activity reduced (0.95 μM) against A549 cell line. Introduction of electron withdrawing groups like trifluoromethyl (**20d**), nitro (**20h**) at para position increased the activity against A549 cell line compared to electron donating substituents like ethyl (**20a**) and methoxy (**20g**). 3,4-di substituted compounds exhibited moderate activity against A549 cell line. 1,2,3-triazole ring at either 1st position or 3rd position on pyrrolo[2,3-b]pyridine did not have much effective on activity against A549 cell line. All the synthesized compounds are exhibiting moderate activity (0.39-1.86 μM) against HeLa cancer cell line. The compound with iodo substitution (**20m**) at ortho position showed better activity (0.13 μM) against MDA MB-231 cancer cell line compared with fluoro (**20j**), chloro (**20k**), bromo (**20l**) and nitro (**20o**) groups at ortho positions, **20e** with electron withdrawing chloro substitution at para position showed potent activity (0.13 μM) than other halo substitutions and also electron donating groups like methoxy (**20g**). 3,4-dimethoxy substitutional compound showed better activity (0.14 μM) than 3,4-dimethyl, 3,4-dichloro, 3,4-difluoro compounds against MDA MB-231 cancer cell line. 1,2,3-triazole ring at 3rd position on pyrrolo[2,3-*b*]pyridine analogues from **22a-k** showed good to moderate activity. Compound with electron withdrawing fluoro at para position (**22j**) showed good activity against all three cell lines A549, HeLa, MDA MB-231 respectively with GI_{50} of 0.18 μM, 0.7 μM, 0.25 μM compared with electron donating substitutions at para position like methoxy (**22k**), methyl (**22b**), tertiary butyl (**22c**) and the unsubstituted one (**22a**). Based on the SAR, we note that the halo groups like fluoro, chloro, iodo at ortho and para positions play a crucial role in antiproliferative activity.

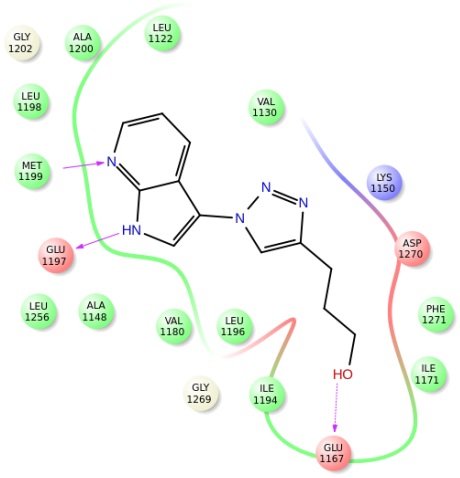
5.2.3. Molecular docking studies

The molecular docking studies of **20a-u** and **22a-k** were performed using ALK (Human anaplastic lymphoma kinase) enzyme using Schrödinger suite 2013. Crystal co-ordinates for ALK (Human anaplastic lymphoma kinase) were taken from Protein Data Bank (PDB ID: 2XP2). Docking studies were performed using GLIDE, module of Schrödinger. Docking scores by standard precision (Glide-SP) docking were shown in **Table 5.1**. Molecular docking studies revealed that these compounds (**22f**, **22g** and **22i**) bind to the crizotinib binding site of the human anaplastic lymphoma kinase with a binding affinity of -8.318, -8.18 and -8.663, respectively, compared to crizotinib -8.123). The hydroxyl group of **22f**, **22g** and **22i** showed hydrogen bonding interaction with LYS 1150, ASP 1270 and GLU 1167 amino acids. This orientation is

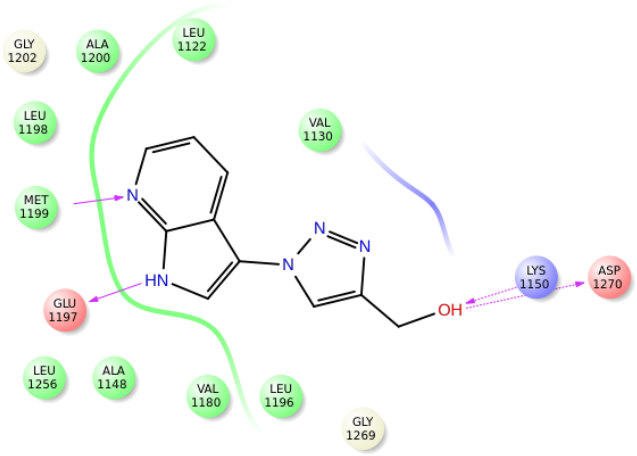
fruitful for extensive interactions such as hydrophobic interactions (**Figure 5.3**). Therefore, substitution with hydroxyl group in **22f**, **22g** and **22i** resulted in improved docking score, which contributed for the antiproliferative activity. Amino acid interaction pattern of active compounds **22f**, **22g** and **22i** are shown in **Figure 5.3** along with crizotinib (PF-02341066) as standard. Crizotinib has shown docking score of -8.123.



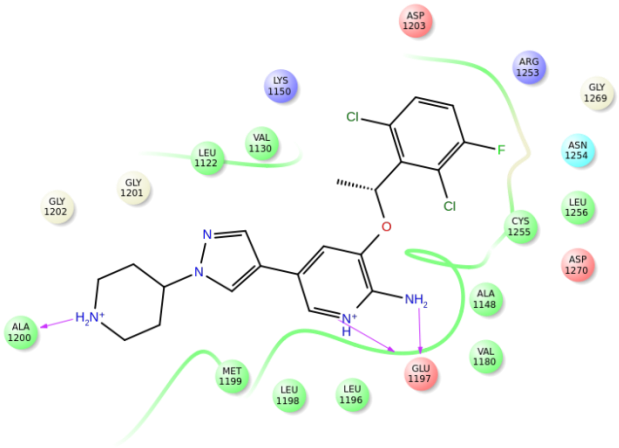
22f



22g

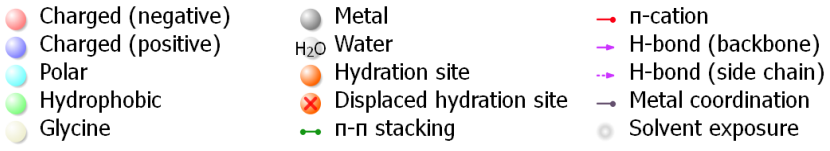


22i



Crizotinib

Figure 5.3: Amino acid interaction pattern of **22f, 22g, 22i** and **crizotinib**



5.2.4. DNA binding affinity

DNA binding affinity between synthesized compound (**20d**) and CtDNA was studied with UV-visible and fluorescence spectroscopes.

5.2.4.1.UV- Visible spectra studies

UV–visible spectroscopy is frequently used technique to discover the interaction studies between biological macromolecules and small molecules. We used UV–visible spectroscopy to investigate the absorbance spectra of **20d**-CtDNA interaction (**Figure 5.4**). The characteristic peak of compound **20d** alone was observed near 222nm. However, on subsequent addition of CtDNA to compound **20d**, the absorbance of compound gradually decreased, indicating hypochromic effect. Hypochromic effect interaction of compound **20d** with CtDNA indicates strong intermolecular interaction. This hypochromic effect is due to the overlap of the electron cloud of the compound **20d** with the CtDNA base pairs [48, 49]. Hypochromic effect in UV–visible spectra upon compound binding to CtDNA is a characteristic of an intercalating binding mode [50, 51].

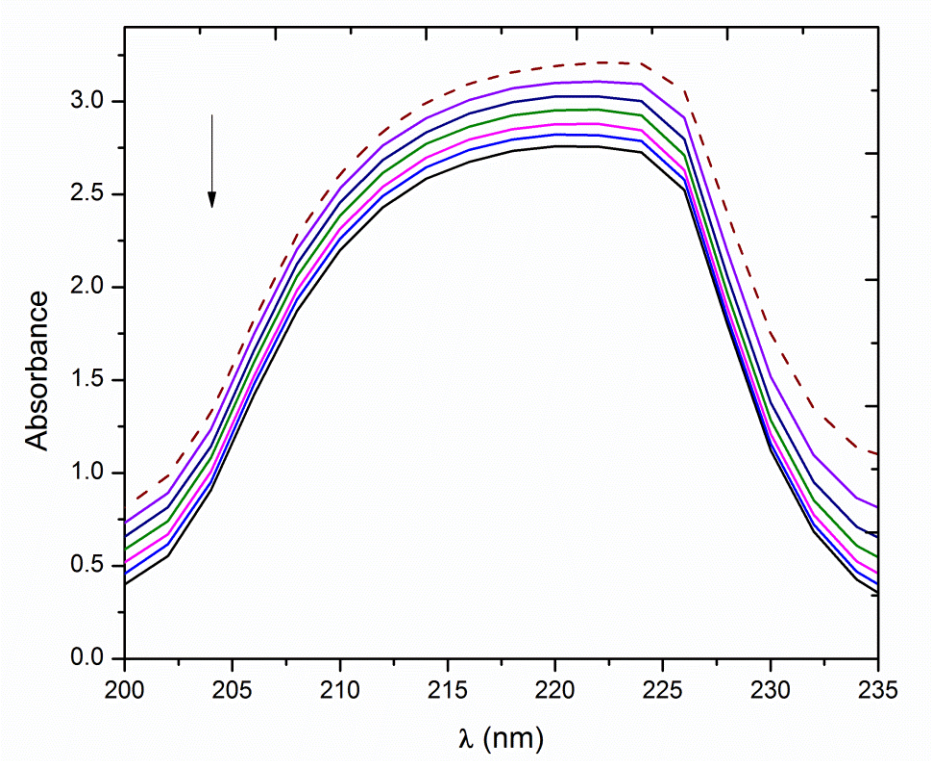


Figure 5.4: The absorption spectra of Compound **20d**-CtDNA system: nm, [Compound] = 0.015× 10^{-5} M. Arrow shows the absorption intensity changes upon increasing CtDNA concentration

The intrinsic binding constant K_b of the compound to CtDNA was determined from following equation.

$$[DNA]/|\varepsilon_a - \varepsilon_f| = [DNA]/|\varepsilon_b - \varepsilon_f| + 1/K_b|\varepsilon_b - \varepsilon_f$$

Here [DNA] represents the concentration of DNA in base pairs, and ε_a, ε_f and ε_b the apparent extinction coefficient (A_{obs}/[M]), the extinction coefficient for free metal complex (M), and the extinction coefficient for the free metal complex (M) in the fully bound form, respectively. K_b is the equilibrium binding constant (in M^{-1}) of compound binding to DNA. In plots of $[DNA]/\varepsilon_a - \varepsilon_f$ Vs [DNA], K_b is obtained by the ratio of slope to intercept (**Figure 5.5**). The binding constant K_b for compound **20d** is 7.16 $\times 10^4$ M^{-1}. These results indicate that the binding strength of compound **20d** is good through the intercalate mode.

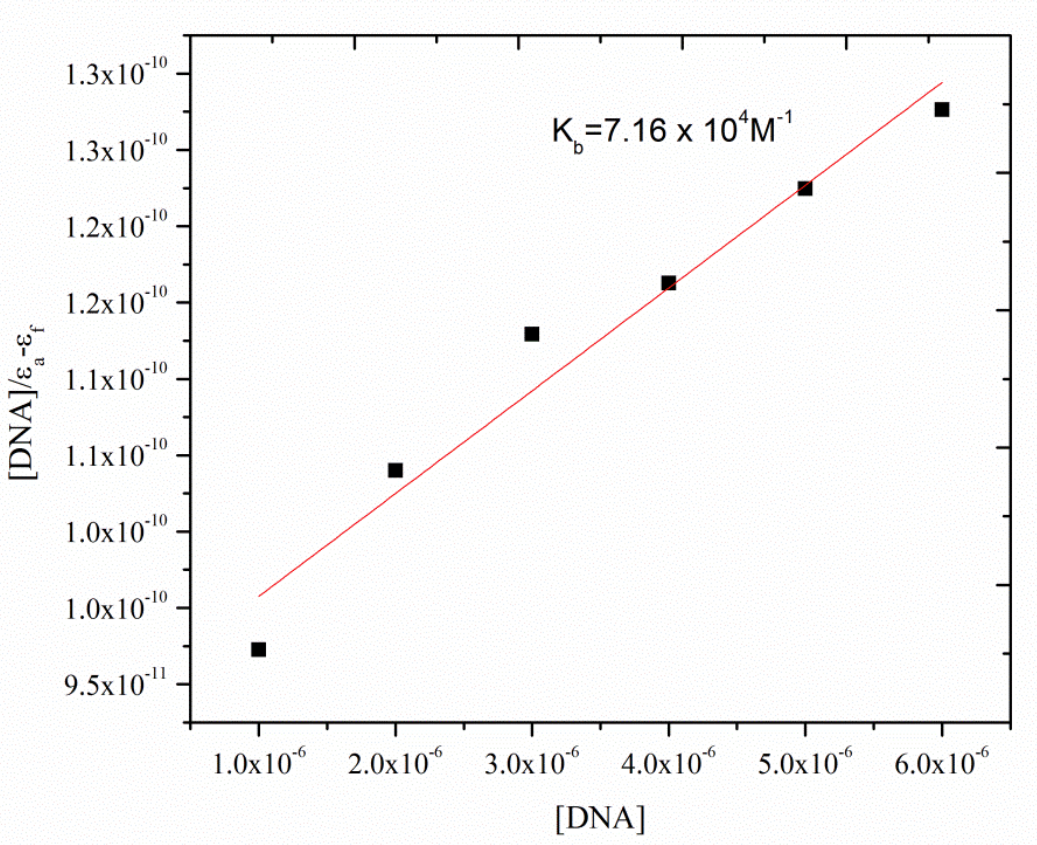


Figure 5.5: Plot of [DNA]/(εa − εf) *vs* [DNA] for the titration of DNA with compound **20d** and solid line is linear fitting of the data

5.2.4.2. Fluorescence spectral studies

The compound **20d** has no fluorescence at room temperature, so the binding of the compound with CtDNA can't be predicted directly through the emission spectra. The spectroscopic changes of Ethidium bromide (EB) on its binding to CtDNA are frequently utilized to study the interaction between CtDNA and new substances such as synthesized molecule [52, 53]. EB displays very feeble fluorescence in the aqueous solution, but in the presence of DNA it exhibits strong fluorescence because of the intercalation to the base pairs in DNA. Intensity of the EB-DNA adduct allows us to determine the affinity of the binding mode of compound **20d** for DNA. If compound can replace EB from EB-DNA, the fluorescence of the solution will be quenched as the free EB molecules are readily quenched by the adjacent water molecules [54, 55]. The fluorescence quenching of EB-CtDNA by the compound **20d** is shown in **Figure 5.6.**

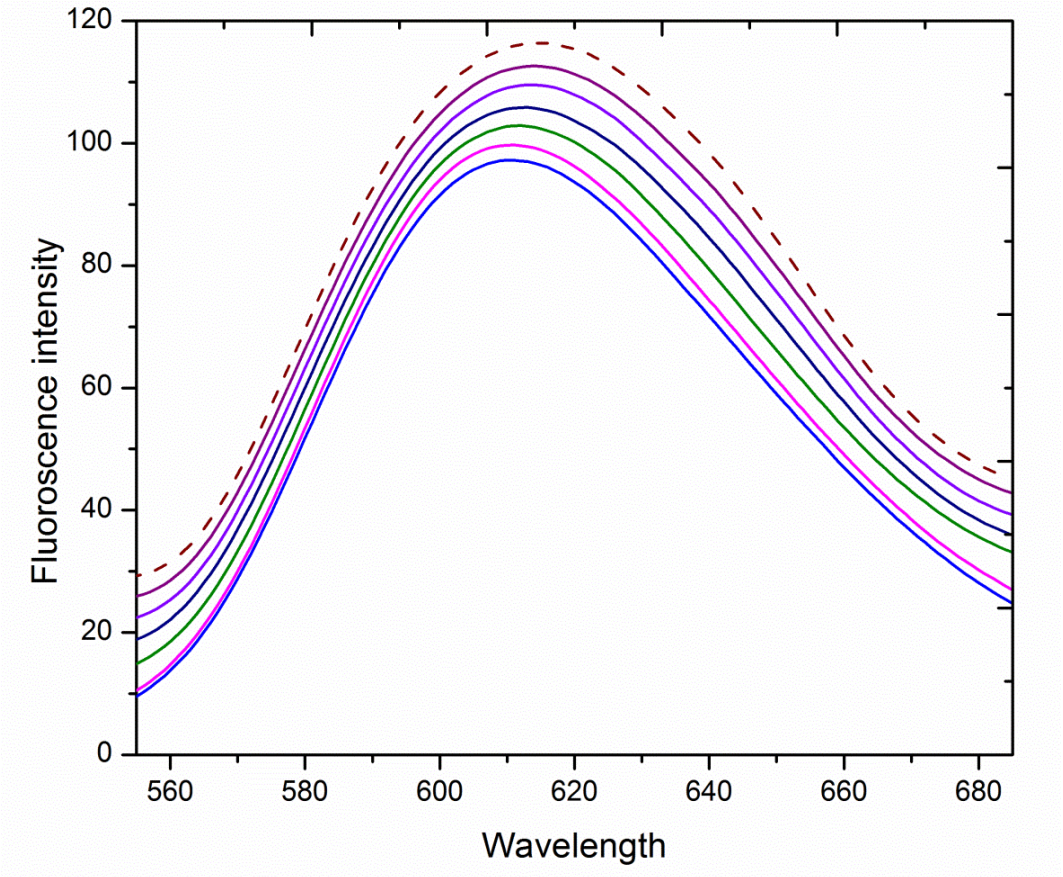


Figure 5.6: The fluorescence spectra of DNA-EB system: ex =500nm, em = 520-720 nm, [Compound] = 0-1.64 × 10^{-5} M. CtDNA(------line). Arrow shows the emission intensity changes upon increasing compound (**20d**) concentration.

The quenching of EB-CtDNA by the compound **20d** is in good agreement with the linear Stern-Volmer equation, which provides further evidence that **20d**, binds to DNA.

$$\frac{I_0}{I} = 1 + K_{sv}\,[Q]$$

In the above equation I_0 is the emission intensity in the absence of quencher, I is the emission intensity in the presence of quencher, K_{sv} is the Stern-Volmer quenching constant, and [Q] is the quencher concentration. The shape of Stern-Volmer plot can be used to characterize the quenching as being predominantly dynamic or static. Plots of I_0/I versus [Q] appear to be linear. The linear relationship of I_0/Iversus [Q] recommends that the quenching result for this system is a static type, means non-fluorescence complex is formed between compound **20d** and CtDNA. K_{sv} is given by the ratio of the slope to the intercept **(Figure 5.7)**. The K_{sv} value for the compound is 5.19×10^{-4} L M^{-1}. This data clearly indicates the interaction of **20d** with CtDNA.

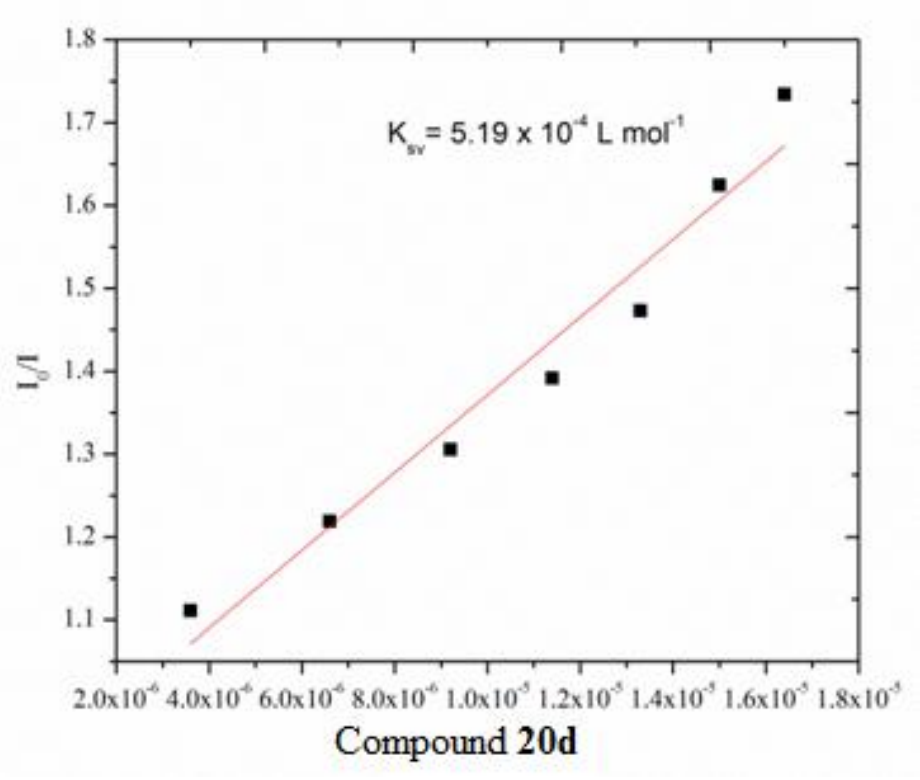


Figure 5.7: Stern-volmer plot of the fluorescence titration data of the compound (**20d**). (Plots of I_0/I versus [Compound **20d**]).

5.3. Conclusion

In summary, a series of pyrrolo[2,3-*b*]pyridine analogues have been designed and synthesized, subsequent by easy reaction protocols. All the synthesized compounds were screened for their growth inhibitory activity against a panel of three different human cancer cell lines such as A549, HeLa and MDA-MB-231. Most of the tested pyrrolo[2,3-*b*]pyridine analogues displayed promising growth inhibitory activity against cancer cell lines. Among all the synthesized compounds, **20c**, **20d**, **20e**, **20h**, **20k**, **20m**, **20n**, **20q**, **20r**, **22f**, **22j**, **22g** and **22k** showed maximum growth inhibitory activity against cancer cell lines at low concentrations. The specific interaction of compound **20d** with calf thymus DNA by intercalate mode, which might further block DNA replication to exert their antiproliferative activity. Our findings from this work with synthesis, antiproliferative activity, molecular modeling and DNA binding experiments demonstrate that this pyrrolo[2,3-*b*]pyridine analogues could be potential candidates for developing cancer diagnostics.

5.4. Experimental section

5.4.1. Chemistry

All reagents were purchased from commercial sources and used with further purification wherever necessary. 7-Aza indole was purchased from Sigma Aldrich. All reactions were monitored by analytical thin layer chromatography (TLC) performed on E-Merck 0.25 mm pre coated silica gel aluminum plates (60 F254) using mixture of pet ether and ethyl acetate. Visualization of the spots on TLC plates was achieved by exposure to UV light. Column chromatography was performed using silica gel (Acme, 100-200mesh). Solvents were dried and purified by distillation prior to use. Solvents for chromatography (Pet ether and ethyl acetate) were distilled prior to use. Evaporations were carried out under reduced pressure on Heidolf rotary evaporator. Melting points were obtained using Stuart SMP30 system and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Avance-III 400MHz (400 MHz for ^{1}H, 100 MHz for ^{13}C), in $CDCl_3$ or DMSO-d_6. Chemical shifts have been expressed in parts per million (δ) relative to tetramethylsilane ($\delta = 0.0$) as an internal standard and coupling constants (J) in Hertz. Low-resolution mass spectra (LC-MS) were recorded on LC/MS-2020 Shimadzu. The UV-Visible absorption spectroscopy was performed on a spectrometer (JASCO model V-650). The

fluorescence spectral titrations were performed on a spectrofluorometer (JASCO model FP-6300). IR spectra were recorded as KBr pellets on Jasco FTIR-4200 spectrometer.

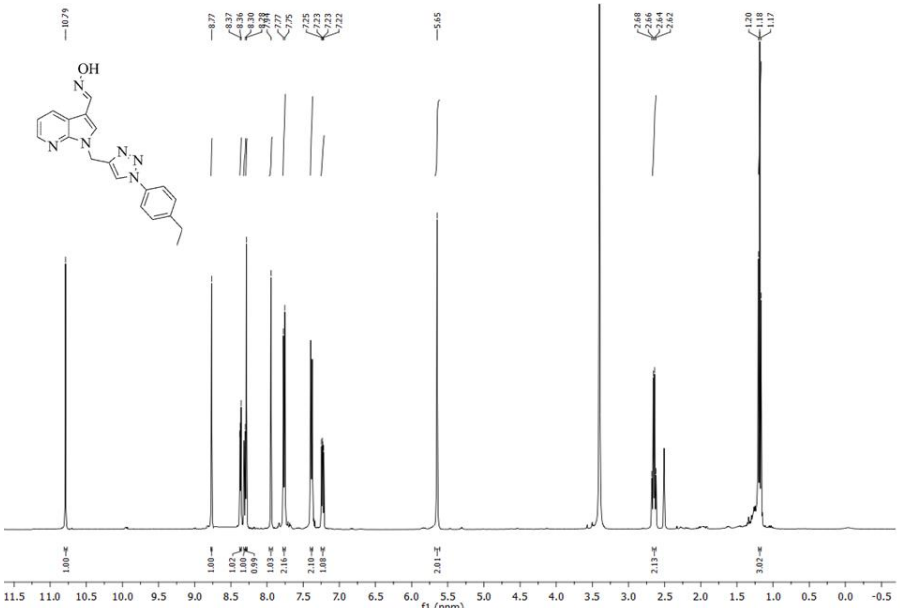
Synthesis of 1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde (17): To a solution of 1*H*-pyrrolo[2,3-*b*]pyridine (1g, 8.5mmol, 1eq) in acetic acid (33%, 15mL), hexamethylenetetramine (HMTA) (1.79g, 9.35mmol, 1.1eq) was added. The reaction mixture was refluxed at 120 °C for 6hrs. Reaction was monitored by TLC and cooled in an ice bath. The resulting precipitate was collected and dried to afford the 1*H*-pyrrolo [2,3-*b*]pyridine-3-carbaldehyde (**17**).

Synthesis of 1-(prop-2-ynyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde (18): To a stirred solution of 1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde (0.0547moles, 1eq) in dimethylformamide (DMF), K_2CO_3 (0.1094moles, 2eq) and propargyl bromide (0.0547moles, 1eq) were added. Reaction mixture was stirred at ambient temperature over night. Reaction was monitored by TLC and water was added to reaction mixture once complete and was followed by extraction with ethyl acetate. Combined organic layers were collected and dried over dry sodium sulphate. Concentrated the organic layer and purified by column chromatography with pet ether and ethyl acetate (15%).

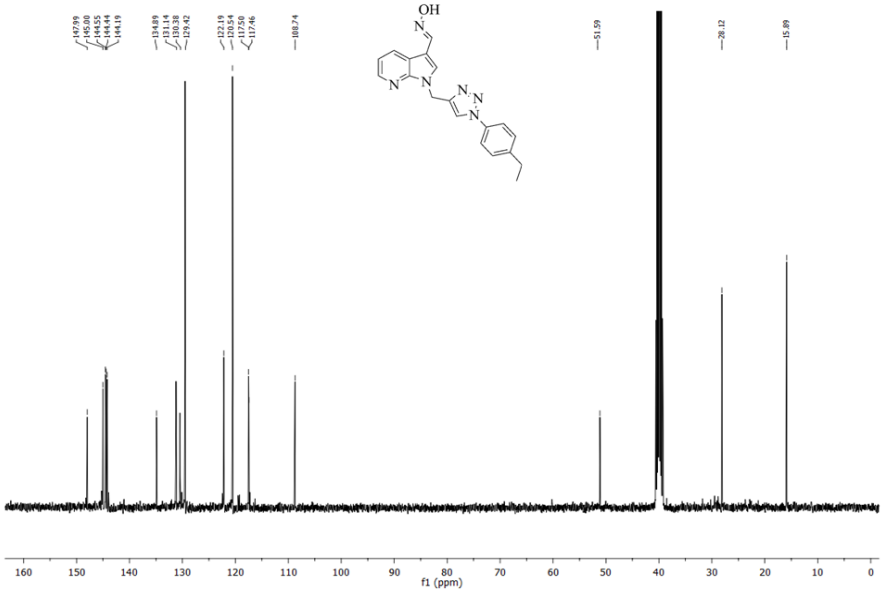
Synthesis of 1-(prop-2-ynyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde oxime (19) :
To a stirred ice cold solution of 1-(prop-2-ynyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbaldehyde (0.02173moles, 1eq) in ethanol, hydroxylamine hydrochloride (0.0260moles, 1.2eq) was added slowly. Sodium hydroxide solution in water was added drop by drop to reaction mixture. Reaction mixture was stirred at room temperature for 2 h. Reaction was monitored by TLC and after completion, as indicated by TLC, acetic acid for neutralization was added to reaction mixture and filtered to get the title compound.

Synthesis of 1-((1-substituted phenyl-1H-1,2,3-triazol-5-yl)methyl)-1*H*-pyrrolo[2,3-*b*] pyridine-3-carbaldehyde oxime (20a-u): To a stirred solution of compound **19** (1.0 mmol) and substituted phenyl azide (1.2 mmol) in ^{t}butanol-water (1:1) (4 mL), $CuSO_4.5H_2O$ (1 mol %) (0.2 mmol) and sodium ascorbate (5 mol %) (0.2 mmol) were added and the reaction mixture was stirred at RT for 12 h. After completion of the reaction, as indicated by TLC, butanol was

removed under reduced pressure. The residue was extracted with ethyl acetate (3 x10 mL) and combined organic layers were collected and washed with saturated brine solution, dried over anhydrous $MgSO_4$ and concentrated in vacuo to get the crude product. The product was further purified by column chromatography using pet ether and ethyl acetae (40%) to afford the title compounds.



^{1}H NMR spectrum of compound **20a**



^{13}C NMR spectrum of compound **20a**

1-((1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20a**)

White solid; yield 65%, 0.33g, m.p. 174-176 °C; IR (KBr, cm^{-1}) 3420, 3010, 1660, 1570, 950. ^{1}H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 8.77 (s, 1H), 8.37 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.34 – 8.29 (m, 1H), 8.28 (s, 1H), 7.94 (s, 1H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 8.6 Hz, 2H), 7.23 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.65 (s, 2H), 2.64 (q, *J* = 7.6 Hz, 2H), 1.18 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 147.99, 145.00, 144.55, 144.44, 144.19, 134.89, 131.14, 130.38, 129.42, 122.19, 120.54, 117.50, 117.46, 108.74, 51.59, 28.12, 15.89. ESI-MS (m/z): calcd. for $C_{19}H_{18}N_6O$ 346.17, found 347.23 $[M + H]^+$.

1-((1-(4-bromo-3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b] pyridine-3-carbaldehyde oxime (**20b**)

Pale yellow solid; yield 76%, 0.53g, m.p. 205-207 °C; IR (KBr, cm^{-1}) 3425, 3015, 1670, 1565, 1130, 950, 575. ^{1}H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 9.01 (s, 1H), 8.36 (d, *J* = 3.8 Hz, 1H), 8.32-8.30 (m, 2H), 8.28 (s, 1H), 8.14 (dd, *J* = 8.7, 2.0 Hz, 1H), 8.08 (d, *J* = 8.7 Hz, 1H), 7.94 (s, 1H), 7.23 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.67 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 147.97, 145.20, 144.37, 144.22, 137.07, 136.34, 131.16, 130.46, 125.70, 122.70, 121.48, 120.06, 120.00, 118.98, 117.53, 117.46, 108.81, 51.61. ESI-MS (m/z): calcd. for $C_{18}H_{12}BrF_3N_6O$ 464.02, found 465.09 $[M + H]^+$.

1-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20c**)

Pale yellow solid; yield 84%, 0.5g, m.p. 166-168 °C; IR (KBr, cm^{-1}) 3462, 3012, 1658, 1568, 953, 595. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.79 (s, 1H), 8.76 (s, 1H), 8.36 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.32 – 8.29 (m, 1H), 8.28 (s, 1H), 7.94 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.41 (d, *J* = 8.6 Hz, 2H), 7.25 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.66 (s, 2H), ^{13}C NMR (100.61 MHz, $CDCl_3$) δ δ 147.95, 145.03, 144.56, 144.43, 144.15, 134.86, 131.12, 130.36, 129.48, 122.12, 120.58, 117.50, 117.46, 108.75, 51.49, ESI-MS (m/z): calcd. for $C_{17}H_{13}BrN_6O$ 396.03, found 397.11 $[M + H]^+$.

1-((1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20d**)

Pale yellow solid; yield 86%, 0.5g, m.p. 178-179 °C; IR (KBr, cm^{-1}) 3435, 3008, 1648, 1572, 1125, 952. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.75 (s, 1H), 8.95 (s, 1H), 8.36 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.32 – 8.29 (m, 1H), 8.28 (s, 1H), 8.13 (d, *J* = 8.5 Hz, 2H), 7.97 (d, *J* = 8.6 Hz, 2H), 7.94 (s, 1H), 7.24 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.67 (s, 2H), ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 147.95, 145.03, 144.56, 144.43, 144.15, 134.86, 131.12, 130.36, 129.48, 122.12, 121.34, 120.58, 117.50, 117.46, 108.75, 51.48, ESI-MS (m/z): calcd. for $C_{18}H_{13}F_3N_6O$ 386.11, found 387.19 $[M + H]^+$.

1-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20e**)

Pale yellow solid; yield 76%, 0.4g, m.p. 173-175 °C; IR (KBr, cm^{-1}) 3398, 3012, 1652, 1574, 956, 792. ^{1}H NMR (400 MHz, DMSO) δ 10.78 (s, 1H), 8.78 (s, 1H), 8.38 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.34 – 8.29 (m, 1H), 8.28 (s, 1H), 7.93 (s, 1H), 7.77 (d, *J* = 8.5 Hz, 2H), 7.38 (d, *J* = 8.6 Hz, 2H), 7.26 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.65 (s, 2H), ^{13}C NMR (101 MHz, DMSO) δ 147.98, 145.02, 144.54, 144.43, 144.17, 134.88, 131.15, 130.37, 129.45, 122.17, 120.54, 117.50, 117.46, 108.74, 51.56, ESI-MS (m/z): calcd. for $C_{17}H_{13}ClN_6O$ 352.08, found 353.16 $[M + H]^+$.

1-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20f**)

Pale yellow solid; yield 79%, 0.4g, m.p. 116-118 °C; IR (KBr, cm^{-1}) 3430, 3010, 1635, 1565, 1235, 945. ^{1}H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 8.81 (s, 1H), 8.37 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.33 – 8.29 (m, 1H), 8.28 (s, 1H), 7.92 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.6 Hz, 2H), 7.28 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.67 (s, 2H), ^{13}C NMR (101 MHz, DMSO) δ 147.98, 145.08, 144.52, 144.42, 144.19, 134.87, 131.16, 130.34, 129.46, 122.18, 120.53, 117.51, 117.46, 108.74, 51.56, ESI-MS (m/z): calcd. for $C_{17}H_{13}FN_6O$ 336.11, found 337.19 $[M + H]^+$.

1-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20g**)

Brown solid; yield 68%, 0.35g, m.p. 172-173 °C; IR (KBr, cm^{-1}) 3420, 3005, 1665, 1575, 1210, 960. ^{1}H NMR (400 MHz, DMSO) δ 10.73 (s, 1H), 8.69 (s, 1H), 8.37 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.33 – 8.28 (m, 1H), 8.26 (s, 1H), 7.92 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.28 (dd, *J* = 7.8, 4.7 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 2H), 5.63 (s, 2H), 3.81 (s, 3H), ^{13}C NMR (101 MHz, DMSO) δ

147.96, 145.04, 144.54, 144.46, 144.18, 134.86, 131.17, 130.36, 129.46, 122.18, 120.53, 117.51, 117.46, 108.74, 57.45, 51.56, ESI-MS (m/z): calcd. for $C_{18}H_{16}N_6O_2$ 348.13, found 349.21 $[M + H]^+$.

1-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20h**)

Yellow solid; yield 90%, 0.49g, m.p. 199-201 °C; IR (KBr, cm^{-1}) 3440, 3002, 1645, 1565, 1520,1340, 950. ^{1}H NMR (400 MHz, DMSO) δ 10.76 (s, 1H), 8.76 (s, 1H), 8.36 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.34 – 8.28 (m, 1H), 8.27 (s, 1H), 7.94 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 8.6 Hz, 2H), 7.27 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.66 (s, 2H), ^{13}C NMR (101 MHz, DMSO) δ 147.96, 145.06, 144.53, 144.44, 144.16, 134.83, 131.16, 130.38, 129.45, 122.17, 120.54, 117.50, 117.46, 108.74, 51.58, ESI-MS (m/z): calcd. for $C_{17}H_{13}N_7O_3$ 363.11, found 364.18 $[M + H]^+$.

1-((1-(4-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20i**)

Brown solid; yield 87%, 0.58g, m.p. 180-182 °C; IR (KBr, cm^{-1}) 3436, 3009, 1643, 1574, 951, 520. ^{1}H NMR (400 MHz, DMSO) δ 10.77 (s, 1H), 8.76 (s, 1H), 8.38 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.34 – 8.29 (m, 1H), 8.28 (s, 1H), 7.93 (s, 1H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.6 Hz, 2H), 7.26 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.67 (s, 2H), ^{13}C NMR (101 MHz, DMSO) δ 147.93, 145.11, 144.58, 144.42, 144.18, 134.83, 131.16, 130.38, 129.45, 122.17, 120.54, 117.51, 117.46, 108.72, 51.56, ESI-MS (m/z): calcd. for $C_{17}H_{13}IN_6O$ 444.02, found 445.11 $[M + H]^+$.

1-((1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20j**)

Pale yellow solid; yield 87%, 0.58g, m.p. 165-166 °C; IR (KBr, cm^{-1}) 3423, 3005, 1639, 1585, 1310, 945. ^{1}H NMR (400 MHz, DMSO) δ 10.80 (s, 1H), 8.60 (s, 1H), 8.38 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.32-8.30(m, 2H), 7.97 (s, 1H), 7.76 (dd, *J* = 7.75 1.4, Hz, 1H,), 7.68 (dd, *J* = 7.67, 1.3 Hz, 1H), 7.61 (td, *J* = 7.7, 1.8 Hz, 1H), 7.55 (td, *J* = 7.6, 1.5 Hz, 1H). 7.24 (dd, *J* = 7.24 Hz, 1H), 5.68 (s, 2H).^{13}C NMR (100.61 MHz, $CDCl_3$) δ 148.21, 147.32, 142.25, 135.32, 133.82, 132.86, 132.02, 131.27, 130.16, 129.03, 128.87, 126.34, 119.73, 119.04, 118.51, 101.17, 52.72, ESI-MS (m/z): calcd. for $C_{17}H_{13}FN_6O$ 336.11, found 337.17 $[M + H]^+$.

1-((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20k**)

Brown solid, yield 76%, 0.4g, m.p. 168-169 °C; IR (KBr, cm^{-1}) 3395, 3002, 1676, 1581, 955, 810. ^{1}H NMR (400 MHz, DMSO) δ 10.80 (s, 1H), 8.60 (s, 1H), 8.38 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.32-8.30(m, 2H), 7.97 (s, 1H), 7.76 (dd, *J* = 7.75, 1.4 Hz, 1H,), 7.68 (dd, *J* = 7.67, 1.3 Hz, 1H), 7.61 (td, *J* = 7.7, 1.8 Hz, 1H), 7.55 (td, *J* = 7.6, 1.5 Hz, 1H). 7.24 (dd, *J* = 7.24, 1.4 Hz, 1H), 5.68 (s, 2H).^{13}C NMR (100.61 MHz, $CDCl_3$) δ 148.00, 144.44, 144.22, 143.63, 134.84, 132.13, 131.18, 130.98, 130.45, 130.13, 128.91, 128.86, 126.25, 117.52, 117.44, 108.70, 51.33, ESI-MS (m/z): calcd. for $C_{17}H_{13}ClN_6O$ 352.08, found 353.17 $[M + H]^+$.

1-((1-(2-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20l**)

Brown solid; yield 87%, 0.51g, m.p. 152-154 °C; IR (KBr, cm^{-1}) 3467, 3043, 1665, 1586, 943, 628. ^{1}H NMR (400 MHz, DMSO) δ 10.78 (s, 1H), 8.56 (s, 1H), 8.38 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.32-8.30(m, 2H), 7.97 (s, 1H), 7.76 (dd, *J* = 7.75, 1.4 Hz, 1H,), 7.68 (dd, *J* = 7.67 Hz, 1.3 1H), 7.61 (td, *J* = 7.7, 1.8 Hz, 1H), 7.55 (td, *J* = 7.6, 1.5 Hz, 1H). 7.24 (dd, *J* = 7.24, 1.3 Hz, 1H), 5.66 (s, 2H).^{13}C NMR (100.61 MHz, $CDCl_3$) δ 148.00, 144.44, 144.22, 143.63, 134.84, 132.13, 131.18, 130.98, 130.45, 130.13, 128.91, 128.86, 126.25, 117.52, 117.44, 108.70, 51.33, ESI-MS (m/z): calcd. for $C_{17}H_{13}BrN_6O$ 396.03, found 397.12 $[M + H]^+$.

1-((1-(2-iodophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20m**)

Pale yellow; yield 78%, 0.52g, m.p.154-156 °C; IR (KBr, cm^{-1}) 3395, 3024, 1654, 1589, 950, 557. ^{1}H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 8.56 (s, 1H), 8.36 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.32-8.29 (m, 2H), 7.97 (s, 1H), 7.76 (dd, *J* = 7.75, 1.4 Hz, 1H,), 7.68 (dd, *J* = 7.67, 1.3 Hz, 1H), 7.61 (td, *J* = 7.7, 1.8 Hz, 1H), 7.55 (td, *J* = 7.6, 1.5 Hz, 1H). 7.24 (dd, *J* = 7.24, 1.3 Hz, 1H), 5.68 (s, 2H).^{13}C NMR (100.61 MHz, $CDCl_3$) δ 148.00, 144.44, 144.22, 143.63, 134.84, 132.13, 131.18, 130.98, 130.45, 130.13, 128.91, 128.86, 126.25, 117.52, 117.44, 108.70, 51.28, ESI-MS (m/z): calcd. for $C_{17}H_{13}IN_6O$ 444.02, found 445.11 $[M + H]^+$.

1-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20n**)

Brown solid; yield 91%, 0.47g, m.p. 148-150 °C; IR (KBr, cm^{-1}) 3425, 3032, 1658, 1570, 1235, 945. ^{1}H NMR (400 MHz, DMSO) δ 10.78 (s, 1H), 8.58 (s, 1H), 8.38 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.32-8.28 (m, 2H), 7.96 (s, 1H), 7.76 (s, 1H), 7.71 (s,1H), 7.68 (d, *J* = 7.25 Hz, 1H), 7.66 (t, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 7.24 Hz, 1H), 5.65 (s, 2H). 3.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO) 148.12, 144.43, 144.21, 143.62, 134.81, 132.72, 131.34, 130.21, 130.62, 130.11, 128.91, 128.12, 126.25, 117.52, 117.44, 108.72, 56.23, 51.28, ESI-MS (m/z): calcd. for $C_{18}H_{16}N_6O_2$ 348.13, found 349.19 $[M + H]^+$.

1-((1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20o**)

Yellow solid; yield 67%, 0.36g, m.p. 189-190 °C; IR (KBr, cm^{-1}) 3420, 3081, 1685, 1580, 1512, 1325, 940. ^{1}H NMR (400 MHz, DMSO) δ 10.75 (s, 1H), 8.69 (s, 1H), 8.37 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.32-8.29 (m, 1H), 8.28 (s,1H), 8.21 (d, *J* = 7.75 Hz, 1H), 7.93 (s, 1H), 7.90 (d, *J* = 7.75 Hz, 1H,), 7.82 (dd, *J* = 7.6, 1.5 Hz, 2H). 7.24 (dd, *J* = 7.24, 1.4 Hz, 1H), 5.67 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 147.99, 144.39, 144.25, 138.01, 134.82, 131.65, 131.14, 130.46, 129.44, 128.07, 125.96, 125.39, 119.20, 117.54, 117.44, 108.78, 51.60. ESI-MS (m/z): calcd. for $C_{17}H_{13}N_7O_3$ 363.11, found 364.19 $[M + H]^+$.

1-((1-(3-chloro-4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20p**)

Brown solid; yield 87%, 0.48g, m.p. 186-188 °C; IR (KBr, cm^{-1}) 3467, 3015, 1651, 1578, 1125, 958, 814. ^{1}H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 8.83 (s, 1H), 8.36 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.30 (dd, *J* = 7.8, 1.6 Hz, 1H), 8.28 (s, 1H), 8.18 (dd, *J* = 6.4, 2.7 Hz, 1H), 7.95 – 7.91 (m, 2H) 7.63 (t *J* = 6.0 Hz, 1H), 7.23 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.66 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 147.98, 144.98, 144.39, 144.22, 133.95, 131.18, 130.46, 122.83, 122.62, 121.39, 121.08, 118.63, 118.40, 117.52, 117.45, 108.78, 51.62. ESI-MS (m/z): calcd. for $C_{17}H_{12}ClFN_6O$ 370.07, found 371.13 $[M + H]^+$.

1-((1-(3,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20q**)

Pale yellow solid; yield 81%, 0.47g, m.p. 178-180 °C; IR (KBr, cm^{-1}) 3410, 3080, 1660, 1590, 960, 825. ^{1}H NMR (400 MHz, DMSO) δ 10.76 (s, 1H), 8.81 (s, 1H), 8.38 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.31 (dd, *J* = 7.8, 1.6 Hz, 1H), 8.26 (s, 1H), 8.18 (dd, *J* = 6.4, 2.7 Hz, 1H), 7.95 – 7.91 (m, 2H) 7.62 (t, *J* = 6.0 Hz, 1H), 7.21 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.64 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 148.12, 144.91, 144.32, 144.22, 133.94, 131.12, 130.65, 122.75, 122.21, 121.39, 121.08, 118.63, 118.40, 117.52, 117.15, 108.72, 51.64. ESI-MS (m/z): calcd. for $C_{17}H_{12}Cl_2N_6O$ 386.04, found 387.12 $[M + H]^+$.

1-((1-(3,4-difluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20r**)

White solid; yield 72%, 0.38g, m.p. 168-169 °C; IR (KBr, cm^{-1}) 3395, 3029, 1651, 1583, 1280, 954, ^{1}H NMR (400 MHz, DMSO) δ 10.78 (s, 1H), 8.84 (s, 1H), 8.36 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.30 (dd, *J* = 7.8, 1.6 Hz, 1H), 8.27 (s, 1H), 8.19 (dd, *J* = 6.4, 2.7 Hz, 1H), 7.95 – 7.91 (m, 2H) 7.62 (t, *J* = 6.0 Hz, 1H), 7.23 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.65 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 148.14, 144.92, 144.32, 144.22, 133.94, 131.12, 130.65, 122.75, 122.21, 121.41, 121.08, 118.63, 118.40, 117.54, 117.16, 108.74, 51.68. ESI-MS (m/z): calcd. for $C_{17}H_{12}F_2N_6O$ 354.11, found 355.19 $[M + H]^+$.

1-((1-(3,4-dimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20s**)

Brown solid; yield 86%, 0.49g, m.p. 152-153 °C; IR (KBr, cm^{-1}) 3410, 3020, 1650, 1580, 1170, 945, ^{1}H NMR (400 MHz, DMSO) δ 10.74 (s, 1H), 8.71 (s, 1H), 8.37 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.35 – 8.29 (m, 1H), 8.27 (s,1H), 7.93 (s, 1H), 7.66 (d, *J* = 2.0 Hz, 1H), 7.56 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.23 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.63 (s, 2H), 3.29 (s, 3H), 3.26 (s, 3H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 149.69, 149.32, 147.98, 144.43, 144.37, 144.20, 131.17, 130.46, 130.38, 122.37, 117.51, 117.44, 112.66, 112.35, 108.72, 105.09, 56.31, 56.22, 51.61, ESI-MS (m/z): calcd. for $C_{19}H_{18}N_6O_3$ 378.14, found 379.22 $[M + H]^+$.

1-((1-(3,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20t**)

Brown solid; yield 75%, 0.39g, m.p. 188-190 °C; IR (KBr, cm^{-1}) 3420, 3038, 1657, 1579, 943. ^{1}H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 8.72 (s, 1H), 8.37 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.32 – 8.30 (m, 1H), 8.28 (s,1H), 7.94 (s, 1H), 7.66 (d, *J* = 2.0 Hz, 1H), 7.56 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.23 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.64 (s, 2H), 2.27 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 147.98, 144.53, 144.44, 144.20, 138.54, 137.52, 134.88, 131.20, 130.95, 130.45, 122.03, 121.30, 117.72, 117.50, 117.45, 108.74, 51.61, 19.83, 19.41. ESI-MS (m/z): calcd. for $C_{19}H_{18}N_6O$ 346.15, found 347.23 $[M + H]^+$.

1-((1-(benzo[d][1,3]dioxol-5-yl)-1H-1,2,3-triazol-4-yl)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carbaldehyde oxime (**20u**)

Brown solid; yield 88%, 0.48g, m.p. 208-210 °C; IR (KBr, cm^{-1}) 3398 3029, 1654, 1586, 1210, 956, ^{1}H NMR (400 MHz, DMSO) δ 10.78 (s, 1H), 8.82 (s, 1H), 8.38 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.31 (dd, *J* = 7.8, 1.6 Hz, 1H), 8.26 (s, 1H), 8.18 (dd, *J* = 6.4, 2.7 Hz, 1H), 7.95 – 7.91 (m, 2H) 7.62 (t *J* = 6.0 Hz, 1H), 7.21 (dd, *J* = 7.8, 4.7 Hz, 1H), 5.67 (s, 2H), 3.91 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 148.65, 144.91, 144.37, 144.22, 133.99, 131.12, 130.63, 122.75, 122.21, 121.39, 121.08, 118.66, 118.40, 117.52, 117.16, 108.72, 58.23, 51.66. ESI-MS (m/z): calcd. for $C_{18}H_{14}N_6O_3$ 362.11, found 363.21 $[M + H]^+$.

Synthesis of 3-bromo-1*H*-pyrrolo [2, 3-*b*] pyridine (21): 1*H*-pyrrolo[2,3-*b*]pyridine **(16)** was dissolved in dimethylformamide (DMF)and added *N*-bromosuccinimide in DMF solution was added to dropwise at ambient temperature. The reaction mixture was stirred overnight at room temperature. Once the reaction is completed as indicated by TLC and water was added to reaction mixture. 3-bromo-1*H*-pyrrolo[2, 3-*b*]pyridine (**21**) was collected by filtration.

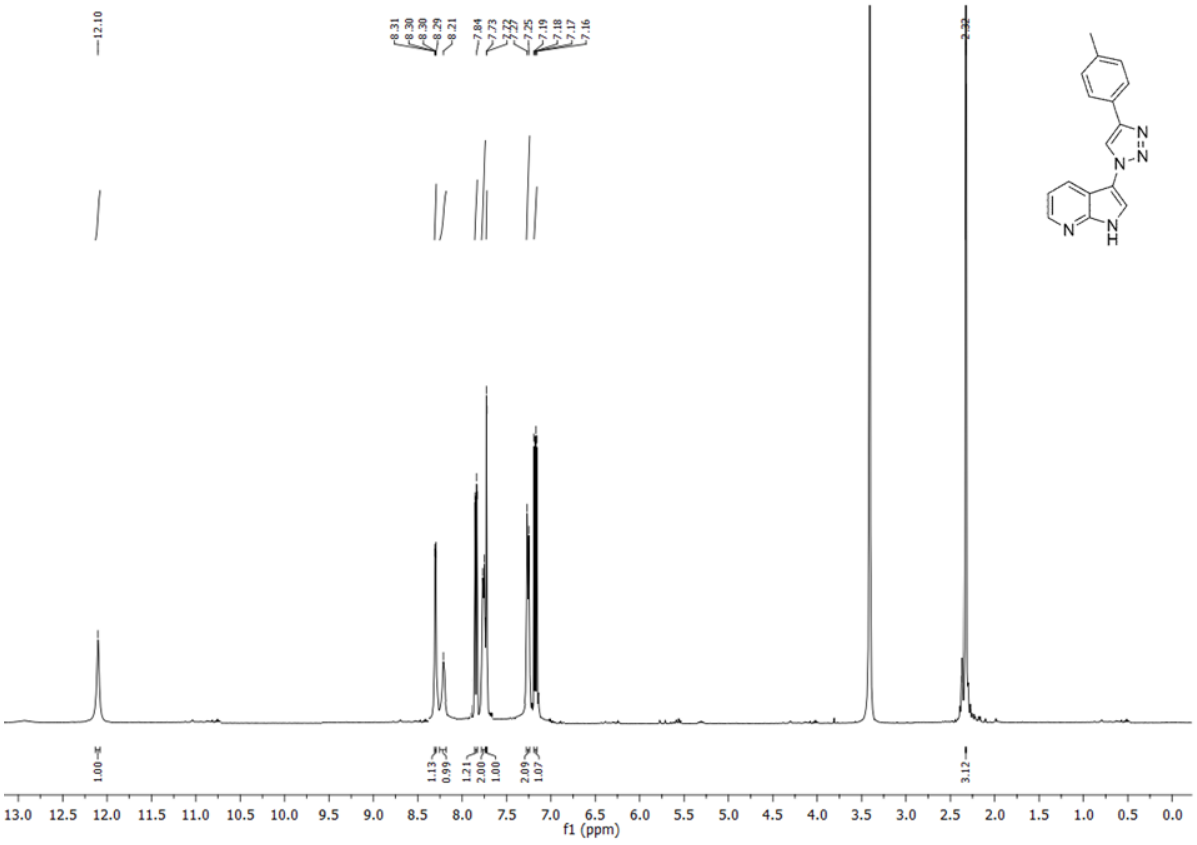
Synthesis of 3-(4-substituted-1H-1,2,3-triazol-1-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (22a-k):

3-bromo-1*H*-pyrrolo[2,3-*b*]pyridine (**21)** (0.5 mmol, 1 equiv) was mixed with a variety of alkynes (0.55 mmol, 1.1 equiv) 9:1 DMSO/H_2O in a 20 mL scintillation vial. To this mixture were added L-proline (0.1 mmol, 0.2 equiv), Na_2CO_3 (0.1 mmol, 0.2 equiv), NaN_3 (0.6 mmol, 1.2 equiv), sodium ascorbate (0.05 mmol, 0.1 equiv) and $CuSO_4.5H_2O$ (0.025 mmol, 0.05 equiv)

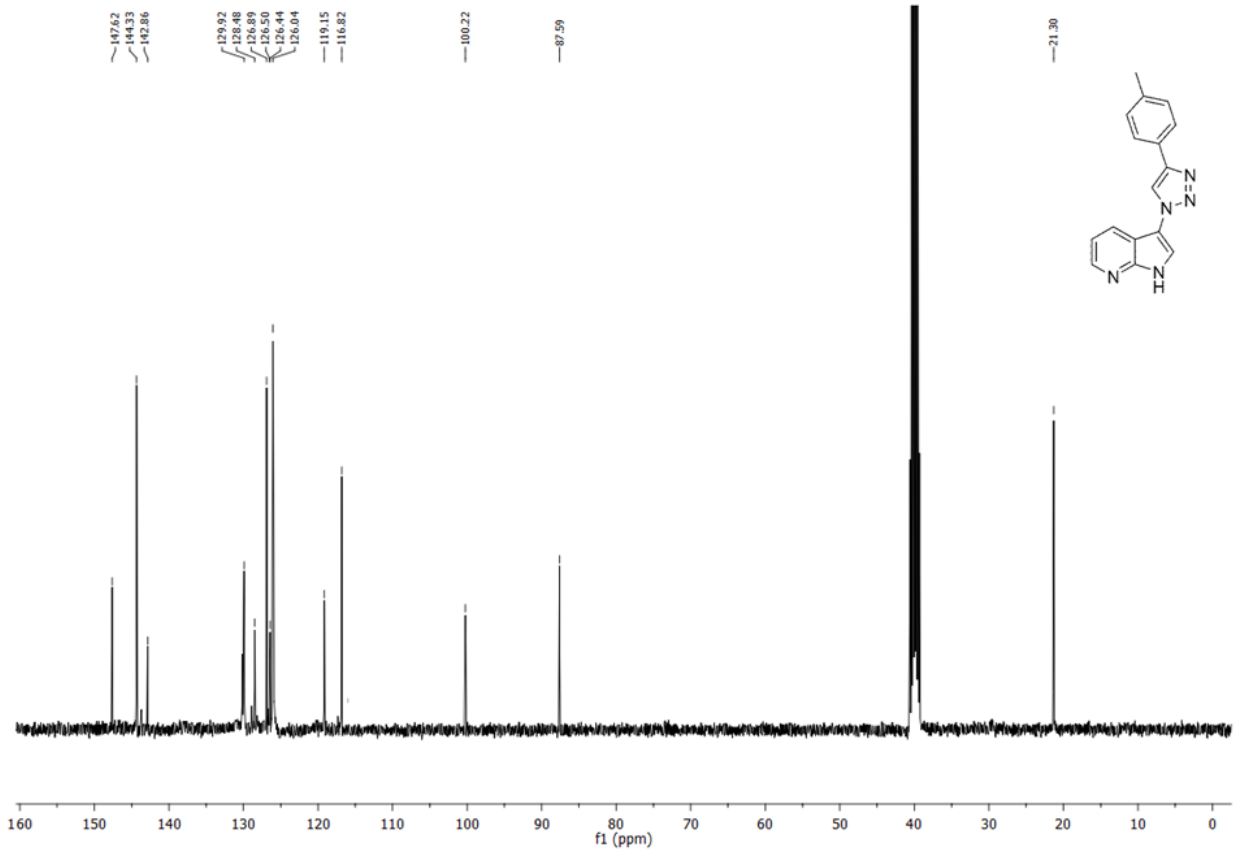
were added. The mixture was stirred overnight at 60 °C. Upon completion of the reaction (monitored by TLC), the crude mixture was poured into water and extracted with ethyl acetate. Combined the organic layers and dried over anhydrous sodium sulphate. Purified by column chromatography with petroleum ether and ethyl acetate (15%).

3-(4-phenyl-1H-1,2,3-triazol-1-yl)-1H-pyrrolo[2,3-b]pyridine (**22a**)

White solid; yield 85%, 0.33g, m.p. 161-163 °C; IR (KBr, cm^{-1}) 3310, 3025, 1655. ^{1}H NMR (400 MHz, DMSO) δ 12.89 (s, 1H), 8.71 (s, 1H), 8.27 (dd, *J* = 4.6, 1.2 Hz, 1H). 8.01 (dd, *J* = 7.9, 1.5 Hz, 2H). 7.55 (t, *J* = 7.2 Hz, 2H). 7.45 (d, *J* = 7.2 Hz, 2H). 7.15 (dd, *J* = 7.9, 4.7 Hz, 1H). 6.82 (s, 1H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.32, 145.92, 142.86, 132.04, 131.66, 129.38, 128.69, 127.91, 126.64, 125.01, 121.21, 116.82, 101.45, ESI-MS (m/z): calcd. for $C_{15}H_{11}N_5$ 261.1, found 262.17 $[M + H]^+$.



^{1}H NMR spectrum of compound **22b**



^{13}C NMR spectrum of compound **22b**

3-(4-p-tolyl-1H-1,2,3-triazol-1-yl)-1H-pyrrolo[2,3-b]pyridine (**22b**)

Pale yellow solid; yield 84%, 0.35g, m.p. 117-119 °C; IR (KBr, cm^{-1}) 3322, 3032, 1663. ^{1}H NMR (400 MHz, DMSO) δ 12.10 (s, 1H), 8.30 (dd, *J* = 4.6, 1.2 Hz, 1H). 8.21 (s, 1H), 7.84 (dd, *J* = 7.9, 1.5 Hz, 1H). 7.75 (d, *J* = 7.2 Hz, 2H). 7.72 (d, *J* = 7.2 Hz, 1H). 7.25 (d, *J* = 4.3 Hz, 2H). 7.17 (dd, *J* = 7.9, 4.7 Hz, 1H). 2.32 (s, 3H), ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.62, 144.33, 142.86, 129.92, 128.48, 127.42, 126.89, 126.50, 126.04, 119.15, 116.82, 100.22, 87.99, 21.30, ESI-MS (m/z): calcd. for $C_{16}H_{13}N_5$ 275.12, found 276.19 $[M + H]^+$.

3-(4-(4-tert-butylphenyl)-1H-1,2,3-triazol-1-yl)-1H-pyrrolo[2,3-b]pyridine (**22c**)

Pale yellow solid; yield 83%, 0.40g, m.p. 121-123 °C; IR (KBr, cm^{-1}) 3328, 3028, 1665. ^{1}H NMR (400 MHz, DMSO) δ 12.07 (s, 1H), 8.30 (dd, *J* = 4.6, 1.2 Hz, 1H), 8.19 (s, 1H), 7.85 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.77 (d, *J* = 7.2 Hz, 2H), 7.71 (d, *J* = 7.2 Hz, 1H), 7.46 (d, *J* = 4.3 Hz, 2H),

7.18 (dd, *J* = 7.9, 4.7 Hz, 1H), 1.30 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 149.26, 147.63, 144.23, 131.14, 131.01, 128.51, 126.89, 126.36, 126.04, 125.86, 119.16, 116.82, 100.15, 34.82, 31.50. ESI-MS (m/z): calcd. for $C_{19}H_{19}N_5$ 317.16, found 317.24 $[M + H]^+$.

3-(4-butyl-1H-1,2,3-triazol-1-yl)-1H-pyrrolo[2,3-b]pyridine (**22d**)

Brown solid; yield 79%, 0.29g, m.p. 156-157 °C; IR (KBr, cm^{-1}) 3325, 3056, 1667, 1472. ^{1}H NMR (400 MHz, DMSO) δ 12.09 (s, 1H), 8.30 (dd, *J* = 4.6, 1.2 Hz, 1H), 7.84 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.76 (s, 1H), 7.19 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.08 (dd, *J* = 7.9, 4.7 Hz, 1H), 2.01 (t, *J* = 6.8 Hz, 3H), 1.55-1.45 (m, 6H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 148.76, 147.36, 144.72, 142.74, 128.60, 126.89, 119.14, 116.87, 101.22, 10.25, 9.29, 8.86, 8.12. ESI-MS (m/z): calcd. for $C_{13}H_{15}N_5$ 241.13, found 242.21 $[M + H]^+$.

3-(4-cyclopropyl-1H-1,2,3-triazol-1-yl)-1H-pyrrolo[2,3-b]pyridine (**22e**)

Pale yellow solid; yield 69%, 0.23g, m.p. 148-149 °C; IR (KBr, cm^{-1}) 3342, 3024, 1651, 1425. ^{1}H NMR (400 MHz, DMSO) δ 12.11 (s, 1H), 8.31 (dd, *J* = 4.6, 1.2 Hz, 1H). 7.85 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.74 (s, 1H), 7.18 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.06 (dd, *J* = 7.9, 4.7 Hz, 1H), 2.00 (tt, *J* = 7.2 Hz, *J* = 6.9 Hz, 1H), 0.92 (t, *J* = 7.2 Hz, 2H), 0.72 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 148.72, 147.62, 144.32, 142.74, 128.60, 126.89, 126.04, 119.16, 116.81, 100.26, 8.90, 8.37, ESI-MS (m/z): calcd. for $C_{12}H_{11}N_5$ 225.1, found 226.19 $[M + H]^+$.

2-(1-(1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-1,2,3-triazol-4-yl)propan-2-ol (**22f**)

Pale yellow solid; yield 87%, 0.32g, m.p. 149-151 °C; IR (KBr, cm^{-1}) 3420, 3336, 3054, 1676, 1450. ^{1}H NMR (400 MHz, DMSO) δ 12.08 (s, 1H), 8.30 (dd, *J* = 4.6, 1.2 Hz, 1H). 7.93 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.83 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.72 (s, 1H), 7.06 (dd, *J* = 7.9, 4.7 Hz, 1H), 6.44 (s, 1H), 1.34 (s, 3H), 1.39 (s, 3H).^{13}C NMR (101 MHz, DMSO) δ 148.86, 147.62, 144.33, 128.48, 126.89, 126.04, 119.15, 116.82, 100.22, 87.59. 32.05 ESI-MS (m/z): calcd. for $C_{12}H_{13}N_5O$ 243.11, found 244.21 $[M + H]^+$.

3-(1-(1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-1,2,3-triazol-4-yl)propan-1-ol (**22g**)

White solid; yield 73%, 0.27g, m.p. 150-152 °C; IR (KBr, cm^{-1}) 3425, 3327, 3055, 1675, 1446. ^{1}H NMR (400 MHz, DMSO) δ 12.10 (s, 1H), 8.31 (dd, *J* = 4.6, 1.2 Hz, 1H), 7.86 (dd, *J* = 7.9,

1.5 Hz, 1H), 7.78 (s, 1H), 7.18 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.09 (dd, *J* = 7.9, 4.7 Hz, 1H), 4.01 (s, 1H), 1.61-1.48 (m, 6H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 148.32, 147.12, 144.62, 142.74, 128.65, 126.82, 119.12, 116.87, 101.22, 11.02, 10.24, 9.31. ESI-MS (m/z): calcd. for $C_{12}H_{13}N_5O$ 243.11, found 244.18 $[M + H]^+$.

Ethyl 1-(1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-1,2,3-triazole-4-carboxylate (**22h**)

Pale yellow solid; yield 78%, 0.30g, m.p. 165-167 °C; IR (KBr, cm^{-1}) 3317, 3062, 1672, 1424. ^{1}H NMR (400 MHz, DMSO) δ 12.08 (s, 1H), 8.30 (dd, *J* = 4.6, 1.2 Hz, 1H), 7.84 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.79 (s, 1H), 7.17 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.06 (dd, *J* = 7.9, 4.7 Hz, 1H), 3.78 (q, *J* = 6.4 Hz, 2H), 2.41(t, *J* = 6.2 Hz 3H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 152.21, 148.31, 147.32, 144.48, 142.52, 128.61, 126.82, 119.13, 116.82, 101.81, 62.32, 15.32. ESI-MS (m/z): calcd. for $C_{12}H_{11}N_5O_2$ 257.09, found 258.17 $[M + H]^+$.

(1-(1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-1,2,3-triazol-4-yl)methanol (**22i**)

White solid; yield 83%, 0.27g, m.p. 120-121 °C; IR (KBr, cm^{-1}) 3435, 3325, 3020, 1670.^{1}H NMR (400 MHz, DMSO) δ 12.11 (s, 1H), 8.28 (dd, *J* = 4.6, 1.2 Hz, 1H), 7.84 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.77 (s, 1H), 7.19 (dd, *J* = 7.9, 4.7 Hz, 1H), 7.08 (dd, *J* = 7.9, 4.7 Hz, 1H), 4.12 (s, 1H), 3.12 (s, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 148.32, 147.12, 144.62, 142.74, 128.65, 126.82, 119.12, 116.87, 101.22, 56.43. ESI-MS (m/z): calcd. for $C_{10}H_9N_5O$ 215.08, found 216.17 $[M + H]^+$.

3-(4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)-1H-pyrrolo[2,3-b]pyridine (**22j**)

Brown solid; yield 69%, 0.29g, m.p. 127-128 °C; IR (KBr, cm^{-1}) 3342, 3050, 1671, 1260. ^{1}H NMR (400 MHz, DMSO) δ 12.10 (s, 1H), 8.31 (dd, *J* = 4.6, 1.2 Hz, 1H). 8.20 (s, 1H), 7.86 (dd, *J* = 7.9, 1.5 Hz, 1H). 7.73 (d, *J* = 7.2 Hz, 2H). 7.71 (d, *J* = 7.2 Hz, 1H). 7.23 (d, *J* = 4.3 Hz, 2H). 7.12 (dd, *J* = 7.9, 4.7 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.52, 144.32, 142.84, 129.97, 128.28, 127.12, 126.89, 126.50, 126.16, 119.15, 116.86, 101.22, 100.16. ESI-MS (m/z): calcd. for $C_{15}H_{10}FN_5$ 279.09, found 280.15 $[M + H]^+$.

3-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)-1H-pyrrolo[2,3-b]pyridine (**22k**)

Pale yellow solid; yield 76%, 0.33g, m.p. 165-166 °C; IR (KBr, cm^{-1}) 3325, 3050, 1670, 1175. ^{1}H NMR (400 MHz, DMSO) δ 12.08 (s, 1H), 8.29 (dd, *J* = 4.6, 1.2 Hz, 1H), 8.20 (s, 1H), 7.81 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.79 (d, *J* = 7.2 Hz, 2H), 7.71 (d, *J* = 7.2 Hz, 1H), 7.22 (d, *J* = 4.3 Hz, 2H), 7.18 (dd, *J* = 7.9, 4.7 Hz, 1H), 3.82 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.59, 144.31, 142.82, 129.54, 128.67, 127.40, 126.83, 126.71, 126.04, 119.15, 116.81, 101.21, 100.43, 56.38. ESI-MS (m/z): calcd. for $C_{16}H_{13}N_5O$ 291.11, found 292.18 $[M + H]^+$.

5.4.2. Biology

The cell lines, A549, HeLa and MDA MB 231 (lung, cervical and breast cancer) which were used in this study were procured from American Type Culture Collection (ATCC), United States. The synthesized test compounds were evaluated for their *in vitro* antiproliferative activity in these three different human cancer cell lines. A protocol of 48h continuous drug exposure was used, and a SRB cell proliferation assay was used to estimate cell viability or growth. All the cell lines were grown in Dulbecco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO2 at 37 °C). Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and seeded in 96-well plates in 100 μL aliquots at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5% CO2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs and were incubated for 48 hrs with different doses (0.01, 0.1, 1, 10, ,100μM) of prepared derivatives**.** After 48 hours incubation at 37 °C, cell monolayers were fixed by the addition of 10% (wt/vol) cold trichloroacetic acid and incubated at 4 °C for 1h and were then stained with 0.057% SRB dissolved in 1% acetic acid for 30 min at room temperature. Unbound SRB was washed with 1% acetic acid. The protein –bound dye was dissolved in 10mM Tris base solution for OD determination at 510 nm using a microplate reader (Enspire, Perkin Elmer, USA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

The dose response parameter, GI_{50} was calculated for each experimental agent. Growth inhibition of 50 % (GI_{50}) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug

concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Values were calculated for this parameter if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

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Chapter VI

Synthesis of 1,3,5-triazine analogues as antiproliferative agents

Chapter 6

Synthesis of 1,3,5-triazine analogues as antiproliferative agents

6.1. Introduction

The 1,3,5-triazine scaffold occupies an outstanding position in organic chemistry and medicinal chemistry. It has been broadly used in organic reactions [1-6], due to its specific structure and electronic properties. 1,3,5-triazines have a wide array of biological activities like antiprotozoal [7], anticancer [8-11], antimalarial [12], antiviral [13] and antimicrobial [14,15]. Nitrogen containing triazine heterocycle inhibits the action of an inducible membrane protein which is useful to increase the efflux of the cytotoxic agents and acting at dissimilar targets to varied pharmacological properties [16]. In 1,3,5-triazine at 2-,4- and 6 positions occupied by different reactivity of chlorine atoms, each chlorine is controls by temperature. This phenomenon has amplified interest in this moiety and allows us to the introduce various subtstitutions by replacing the chlorine atoms at various temperature for the preparation of mono-, di- and tri-substituted 1,3,5-triazines [17,18].

Some herbicides like atrazine, cyanazine, simazine, trietazine, and resin modifiers like melamine and benzoguanamine have 1,3,5-triazine as the basic structure [19,20] and there are also drugs containing 1,3,5-triazine nucleus, that are available in the market like Altretamine (antineoplastic agent), Triethylenemelamine (chemotherapy drug) (**Figure 6.1**) [23].

On the other side 3,4,5, trimethoxy substitution enhances the antiproliferative activity. Ursolic acid (UA) is a pentacyclic triterpene and is one of the major efficient elements of many traditional medicine [21]. UA inhibits tumor initiation and promotion and also induces tumor cell differentiation and apoptosis [22]. Several modifications have been introduced in UA and screened for potential antitumor agents. Novel UA derivatives modified at the C-3 and the C-28 positions were designed and synthesized to develop potential antitumor agents.

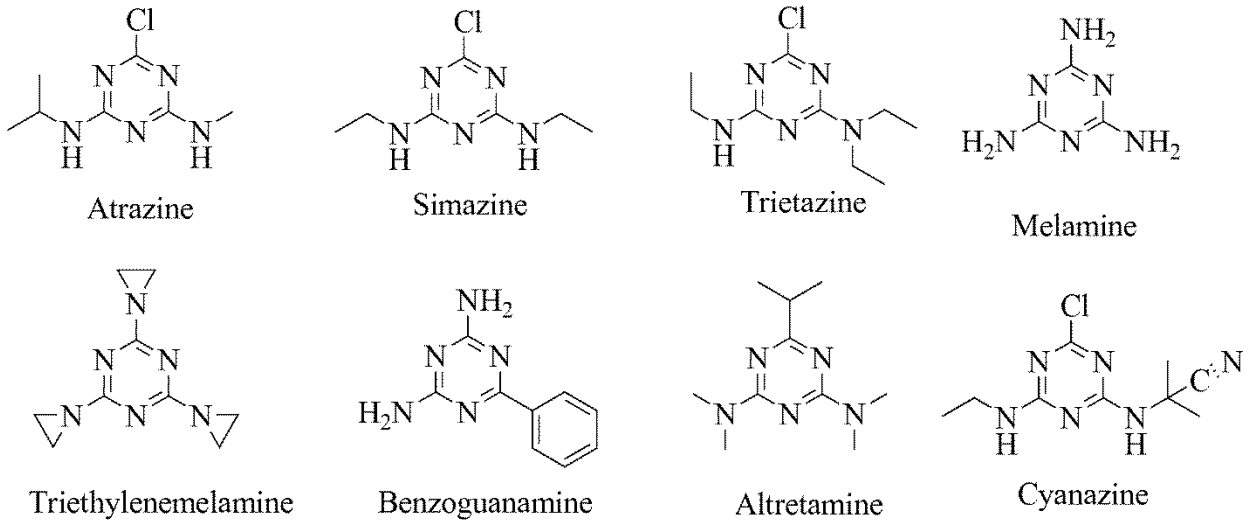


Figure 6.1: Drugs containing 1,3,5-triazine scaffold

Among all UA derivatives trimethoxy substituted analog (**P**) (**Figure 6.2**) exhibited excellent *in vitro* cytotoxicity, induction of cell apoptosis by G1 cell cycle arrest and it induced apoptosis through both of intrinsic and extrinsic apoptosis pathways [23]. Resveratrol is a well-known natural polyphenolic phytoalexin compound [24], its analogs exhibited various cancer chemo-preventive properties, due to their modulation of multiple cellular processes, including apoptosis, cell cycle progression, inflammation, and angiogenesis [25].

A series of trimethoxy derivatives of resveratrol were reported as anticancer agents against various human cancer cell lines [26, 27]. Among these, (*E)*-3,4,5,4-tetramethoxystilbene (**Q**) (**Figure 6.2**) exhibited potent anti-cancer activity and it was active by 30 to 100 folds in comparison to resveratrol [28]. Trimethoxy substituted heteroaromatic analog (**R**) (**Figure 6.2**) of the resveratrol as showed potent growth inhibition in 85% of the cancer cell lines [29]. Incorporate of an oxadiazole ring to 2-anilinonicotinyl linked sulfonyl hydrazide scaffold (**S**) shows potential antitumor activity that considerably inhibited the tubulin polymerization [30]. Trimethoxy containing anticancer analogs depicted in **Figure 6.2**.

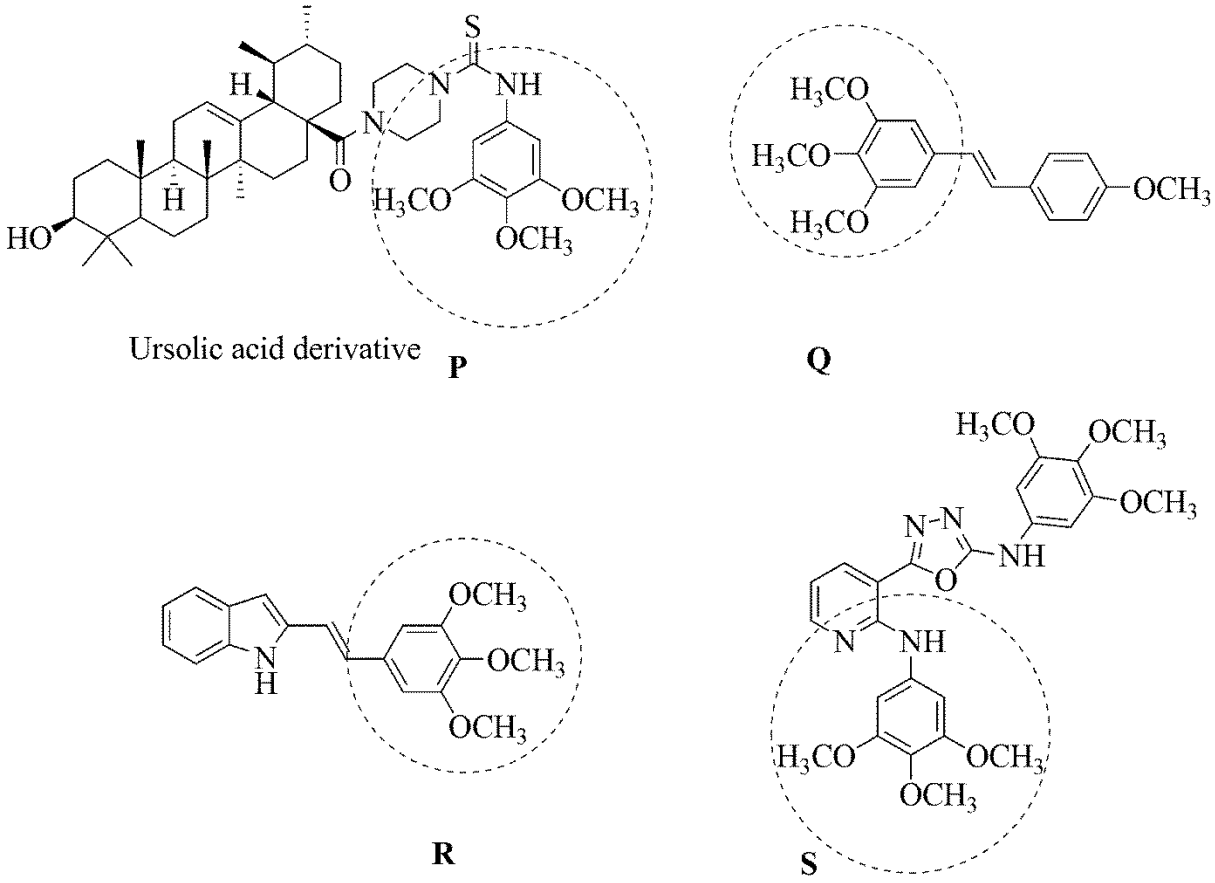


Figure 6.2: Trimethoxy containing anticancer agents

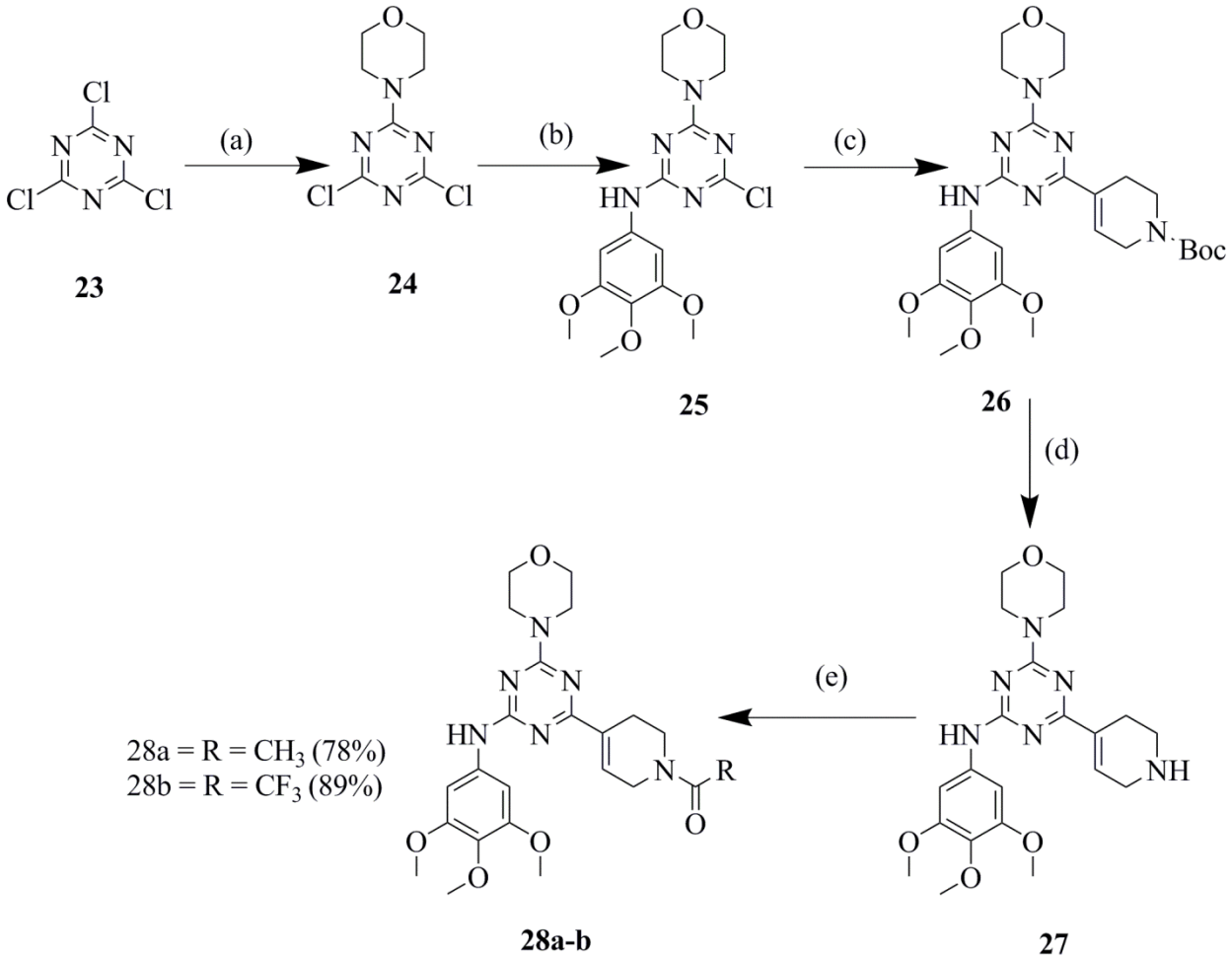
As part of our research program aimed to develop new antiproliferative agents, a series of novel 1,3,5-triazine derivatives were prepared by substituting chlorine atoms of cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) with nucleophilic groups to make a huge diversity of substitutions, according to **Scheme 8** and evaluated the synthesized compounds for their antiproliferative activity.

6.2. Results and Discussion

6.2.1. Chemistry

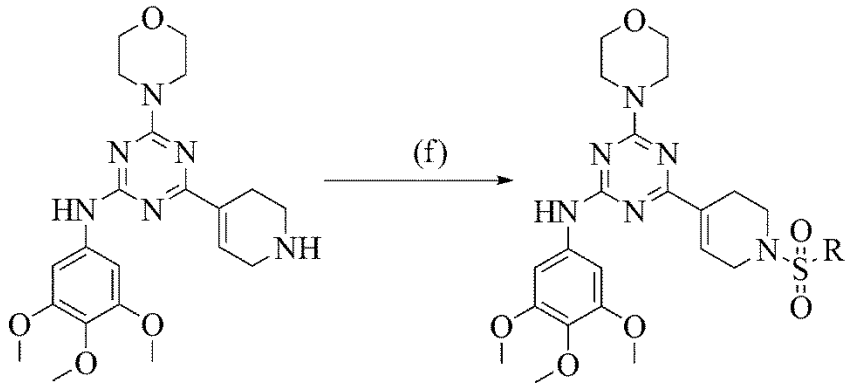
The synthesis of new 1,3,5-triazine analogues is illustrated in **scheme 8**. The synthesis of the substituted 1,3,5-triazines was carried out based on previously reported procedures [31-33]. Cyanuric chloride **23** was reacted with morpholine at 0 °C to give the 1,3-dichloro-5-morpholino triazine **24**, which was reacted with the trimethoxy aniline at RT to give **25**. Subsequently, **25** was converted to **26** by reaction with *N*-Boc-1,2,3,6-tetrahydropyridine-4-boronic acid pinacol

ester under Suzuki conditions. Compound **26** was treated with trifluoro acetc acid and yielded boc deprotected product **27.** Compound **27** on treatment with various anhydrides yielded **28a-b**. Compounds **29a-l** were synthesized by reacting **27** with various sulfonyl chlorides at 0 °C to RT. Compound **27** on treatment with various aliphatic acids and aromatic acids yielded amide products **30a-l.** Compound **27** when treated with various primary and secondary amines yielded uridyl derivatives **31a-k.** All the synthesized compounds were confirmed by ^{1}H, ^{13}C NMR, LCMS and evaluated for their antiproliferative activity.



Scheme 8: Synthetic protocol to achieve the compound (**28a-b**)

Reagents and Conditions: (a) morpholine, Et_3N, Acetone, -20 °C (b) 3,4,5-trimethoxy aniline, DIPEA, 1,4-dioxane, RT (c) *N*-Boc-1,2,5,6-tetrahydropyridine-4-boronic acid pinacol ester, K_2CO_3, $Pd(dppf)Cl_2$, 1,4-dioxane:H_2O, reflux (d) DCM, trifluoro acetic acid, 0 °C-RT (e) acetic anhydrides, Et_3N, DCM, 0 °C-RT



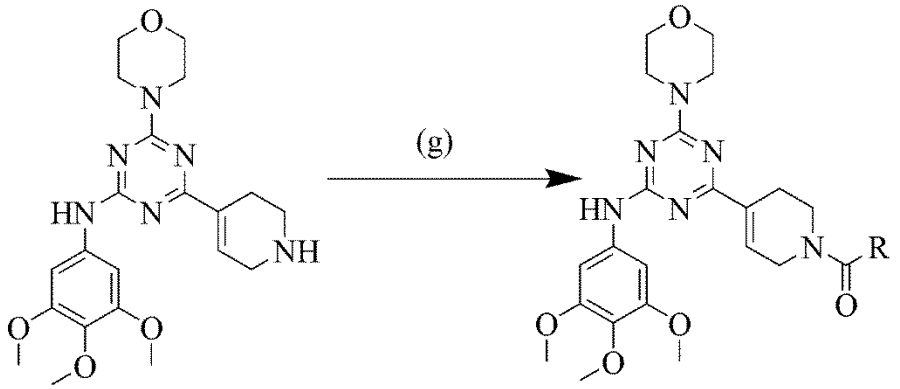
27 **29a-l**

(76-95%)

R=Me, 4-MePh, 4-OMePh, 4-FPh,
4-BrPh, 4-NO_2Ph, 4-CF_3Ph, 4-ClPh,
2-NO_2Ph, 4-$(CH_3)_3$CPh

Scheme 8.1: Synthetic protocol to achieve the compound (**29a-l**)

Reagents and Conditions: (f) various sulfonyl chlorides, Et_3N, DCM, 0 °C-RT



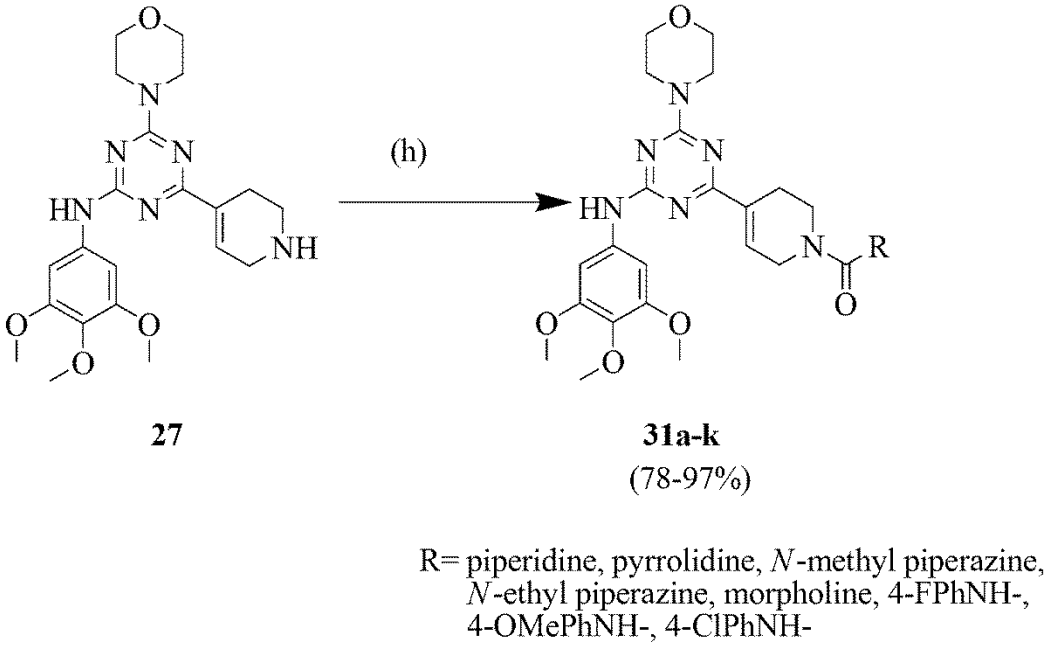
27 **30a-l**

(72-90%)

R= CH_2SH, CH_2CN, 4-ClPh, 4-OMePh
4-BrPh, 2-OH,4-BrPh, 2-Furan, 4-Pyridine

Scheme 8.2: Synthetic protocol to achieve the compound (**30a-l**)

Reagents and Conditions: (g) aromatic, aliphatic acids, EDC.HCl, HOBt, Et_3N, DCM, RT



27 **31a-k** (78-97%)

Scheme 8.3: Synthetic protocol to achieve the compound (**31a-k**)

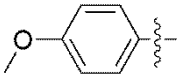
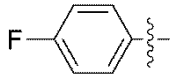
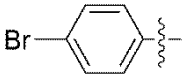
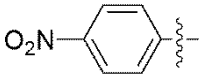
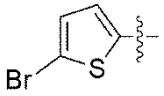
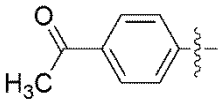
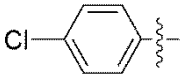
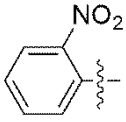
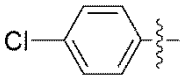
Reagents and Conditions: (h) aromatic, acyclic amines, triphosgene, Et_3N, DCM, 0 °C-RT.

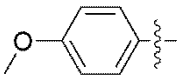
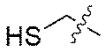
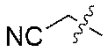
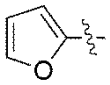
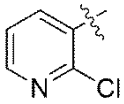
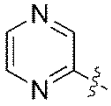
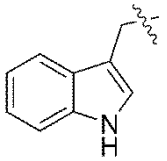
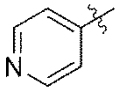
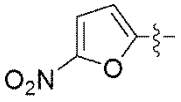
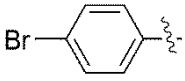
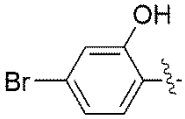
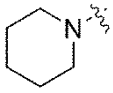
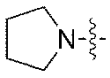
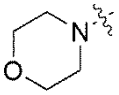
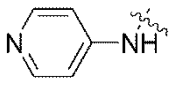
In general ^1H NMR of all the title compounds displayed one broad singlet peak of N-H proton resonated in the range of 7.31-6.97 ppm. A sharp singlet peak due to the alkene proton of tetra hydro pyridine resonated in the range 7.23-6.85 ppm. One singlet resonated in the range of 7.06-6.69 ppm due to protons of trimethoxy phenyl ring. The trimethoxy protons, showed multiplet in the range 3.94-3.78 ppm. A multiplet in the range 3.81-3.61 ppm corresponding to the morpholine protons was observed.

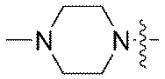
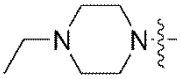
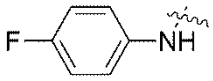
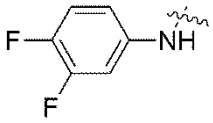
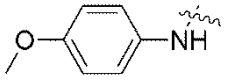
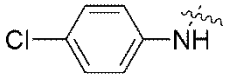
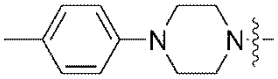
6.2.2. Antiproliferative activity

In vitro antiproliferative activity of the synthesized compounds **28a-b**, **29a-l**, **30a-l** and **31a-k** were evaluated against four types of human cancer cell lines; HeLa (Cervical cancer), HepG2 (liver carcinoma cancer), A549 (Lung cancer), and MCF 7 (Breast cancer) employing sulforhodamine B (SRB) assay method. The minimum inhibition data (expressed as IC_{50}) of synthesized compounds **28a-b**, **29a-l**, **30a-l**, and **31a-k** are shown in **Table 6.1**.

Table 6.1: Antiproliferative activity (IC_{50} in µM) and docking scores of synthesized compounds (**28a-b**, **29a-l**, **30a-l** and **31a-k**)

Entry	R	[a]HeLa	[b]HepG2	[c]A549	[d]MCF 7	Docking Score (SP)
28a	CH_3-	45.7±2.7	50.6±3.7	49.0±1.3	40.2±2.1	-4.316
28b	CF_3-	43.1±2.8	50.6±3.2	28.6±2.9	48.4±3.4	---
29a	CH_3-	32.0±3.2	28.5±1.5	39.0±2.5	28.9±2.9	-3.233
29b		45.7±1.6	25.0±2.9	40.3±3.2	42.1±2.8	---
29c		47.8±3.4	30.6±1.4	34.9±3.8	50.3±1.6	---
29d		50.6±3.2	30.0±1.8	45.6±1.8	29.6±3.5	---
29e		30.6±1.8	40.4±2.3	43.6±1.7	32.5±3.3	---
29f		32.0±2.1	43.2±1.0	34.6±1.5	40.3±2.0	---
29g		32.1±2.8	27.2±1.6	29.9±2.8	40.5±1.8	---
29h		39.6±2.2	28.0±3.2	29.0±1.8	30.6±1.5	---
29i		45.0±3.8	36.5±1.7	32.0±2.6	32.1±2.4	---
29j		51.0±3.7	49.3±1.8	40.8±2.9	30.6±3.2	---
29k		50.4±3.4	48.4±2.9	33.5±3.8	40.4±1.5	---
29l		32.0±2.9	51.0±2.9	33.5±2.5	50.5±2.2	---
30a		34.8±1.9	40.3±2.2	43.5±3.8	38.3±2.7	-4.666

30b		43.0±2.4	30.6±3.3	33.3±2.5	32.1±1.9	---
30c		32.9±1.6	32.9±1.4	43.2±1.6	30.5±2.8	-4.356
30d		34.2±3.8	29.8±2.0	42.0±1.3	40.4±1.6	---
30e		45.0±2.0	37.1±3.2	51.0±1.2	32.0±1.3	---
30f		41.0±2.6	45.0±2.8	42.9±3.4	30.2±2.3	-4.024
30g		48.3±2.7	40.0±2.1	41.9±2.5	39.4±2.8	-3.303
30h		33.2±3.1	32.0±3.6	43.9±2.3	29.8±3.5	---
30i		50.6±3.8	39.5±2.7	48.9±1.8	32.1±2.7	-5.775
30j		**12.3±0.8**	**9.6±0.4**	**10.5±1.0**	**11.7±0.5**	---
30k		40.4±2.9	35.7±3.2	34.0±3.5	39.8±2.5	-5.264
30l		43.9±2.1	38.9±3.5	45.9±3.1	28.0±1.1	-5.68
31a		34.0±2.2	41.7±3.5	30.8±2.1	40.5±2.4	-5.383
31b		29.4±3.5	38.8±1.5	43.3±2.0	34.0±2.9	---
31c		45.2±2.6	29.0±1.3	32.0±1.6	38.2±3.2	-4.65
31d		34.0±2.8	50.0±1.0	32.0±2.3	38.5±1.8	-2.127

31e		40.2±3.1	34.9±1.6	34.6±2.1	40.8±2.6	---
31f		39.5±3.9	45.9±2.8	43.2±2.9	30.0±1.9	---
31g		40.1±2.9	29.1±2.5	45.7±3.7	25.9±2.8	---
31h		30.4±2.2	34.5±1.3	51.0±3.9	30.6±1.9	---
31i		40.5±1.8	43.6±2.4	45.9±2.0	26.4±2.9	---
31j		30.7±1.3	34.9±3.9	35.9±2.6	29.0±3.2	---
31k		39.6±3.2	40.0±3.5	29.6±1.4	49.1±1.7	---
	CA 4	5.8±0.2	4.3±0.3	5.3±0.1	4.2±0.3	
	Nocadazole	1.6±0.1	1.1±0.09	2.3±0.2	1.5±0.2	
	Crizotinib					-8.123

[a]HeLa: Cervical cancer cell line, [b]Hep G2: Liver carcinoma cell line, [c]A549: Lung cancer cell line, [d]MCF 7: Breast cancer cell line, ---:no interaction with ALK enzyme

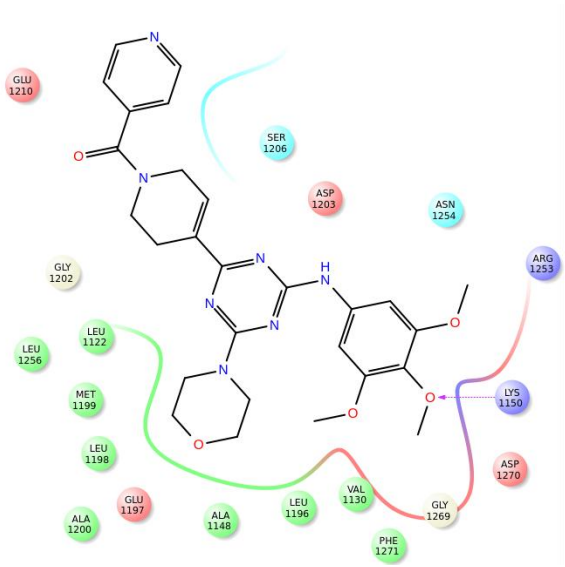
To investigate the cytotoxic activity of these compounds we evaluated the antiproliferative activity on different cancer cell lines like HeLa (Cervical cancer), HepG2 (liver carcinoma cancer), A549 (Lung cancer), and MCF 7 (Breast cancer) and CA4, nocodazole were employed as standard. The concentrations that cause 50% inhibition of cancer cell growth are expressed as IC_{50} values. From the antiproliferative activity results, it is evident that all the synthesized compounds have comparable antiproliferative activity with IC_{50} values ranging from 9.6-51.0μM. Among the thirty seven analogues, compound **30j** showed strong inhibitory effect (IC_{50} = 9.6μM) against HepG2 cell line. Structure-activity relationship studies revealed that there was a significant influence of substituents on tetrahydopyridine ring on cytotoxicity. The compounds **28a-b** with methyl, trifluoromethyl showed moderate activity against all these cancer cell lines. Sulfonamide derivatives **29a-l** with electron donating group like methyl, methoxy, *t*-butyl, electron withdrawing groups like fluoro, chloro, bromo, nitro, trifluoromethyl and hetero

compounds like thiophene did not influence the activity so all these **29a-l** analogs showed moderate activity against the cancer cell lines tested.

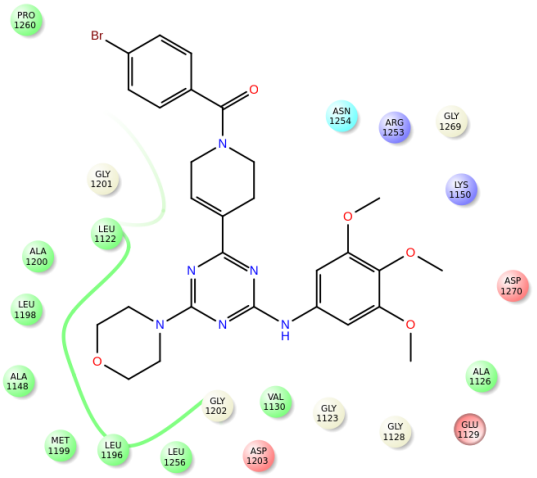
Compounds **30a-l** showed moderate to comparable activity against all cancer cell lines. Compounds with aliphatic substituents like thio (**30c**), cyano (**30d**) are active than aromatic substituents. Electron donating groups like methoxy, electron withdrawing groups like bromo, chloro and heterocyclic compounds like pyridine, indole exhibited moderate activity against all cancer cell lines. Compared with furan derivative (**30e**), nitro furan derivative (**30j**) showed better activity against the tested cancer cell lines. Compound **30j** showed good inhibitory activity (IC_{50} = 9.6μM) against HepG2 cell line compared with other cancer cell lines. Uridyl linkage derivatives (**31a-k**) exhibited moderate activity against all cancer cell lines. Compound with pyrrolidine substituent (**31b**) exhibited better activity than piperidine (**31a**), morpholine (**31c**), methyl piperazine (**31e**), ethyl piperazine (**31f**) derivatives against HeLa cancer cell line. Therfore based on the the SAR study modifications on amide derivates are essential for developing promising anticancer agents.

6.2.3. Molecular docking studies

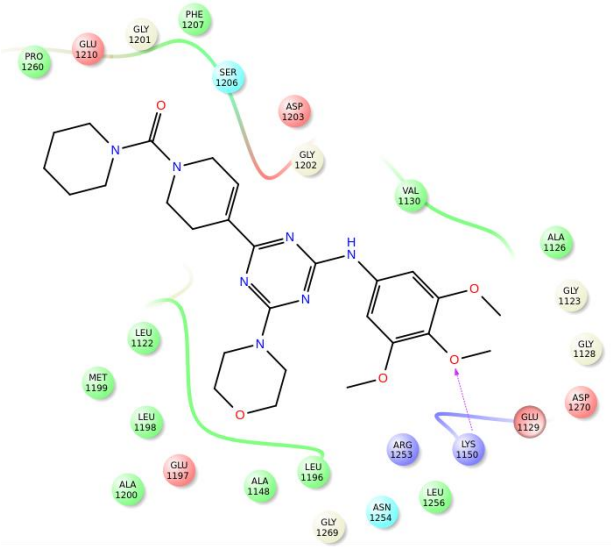
The molecular docking studies of **28a-b**, **29a-l**, **30a-l** and **31a-k** were performed using ALK (Human anaplastic lymphoma kinase) enzyme using Schrödinger suite 2013. Crystal co-ordinates for ALK (Human anaplastic lymphoma kinase) were taken from Protein Data Bank (PDB ID: 2XP2). Docking studies were performed using GLIDE, module of Schrödinger. Docking scores by standard precision (Glide-SP) docking were shown in **Table 6.1**. Molecular docking studies revealed that these compounds (**30i, 30k** and **31a**) bind to the crizotinib binding site of the human anaplastic lymphoma kinase with binding affinity of -5.775, -5.264 and -5.383, respectively, compared to crizotinib -8.123). The trimethoxy group of **30i** and **31a** showed hydrogen bonding interaction with LYS 1150 amino acids. This orientation is fruitful for extensive interactions such as hydrophobic interactions (**Figure 6.3**). Therefore, substitution with trimethoxy group in **30i** and **31a** resulted in improved docking score, which contributed for the antiproliferative activity. Amino acid interaction pattern of active compounds **30i, 30k** and **31a** are shown in **Figure 6.3** along with crizotinib (PF-02341066) as standard. Crizotinib has shown docking score of -8.123.



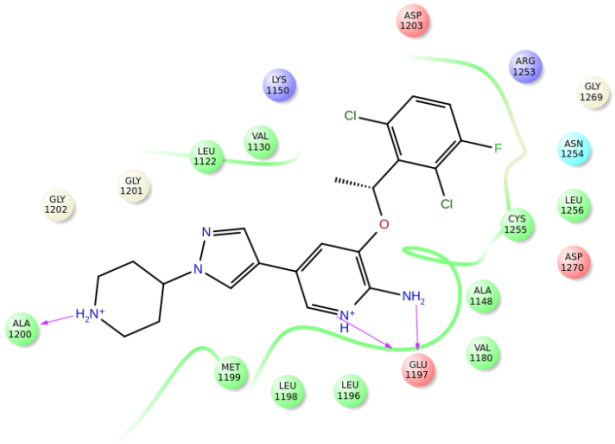
Compound 30i



Compound 30k

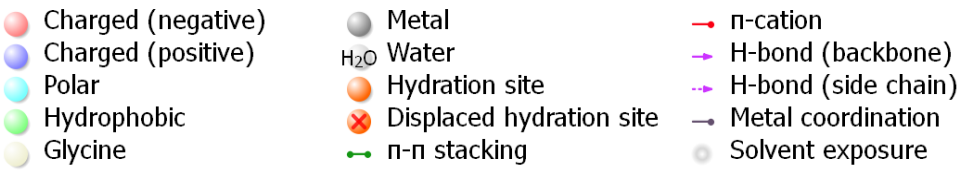


Compound 31a



Crizotinib

Figure 6.3: Amino acid interaction pattern of **30i, 30k, 31a** and **crizotinib**



6.3. Conclusion

In summary, a series of 1,3,5-triazine analogues have been synthesized and screened for their inhibitory activity against a panel of four different human cancer cell lines such as HeLa, HepG2, A549 and MCF-7. Most of the tested 1,3,5-triazine analogues displayed promising inhibitory activity against cancer cell lines. Among all the synthesized compounds **30j** showed potent activity against the cancer cell lines tested at low concentrations. Our findings from this work with synthesis, antiproliferative activity and molecular modeling experiments demonstrate that 1,3,5-triazine analogues could be potential candidates for developing anticancer agents.

6.4. Experimental section

6.4.1. Chemistry

All reagents were purchased from commercial sources and used with further purification wherever necessary. All reactions were monitored by analytical thin layer chromatography (TLC) performed on E-Merck 0.25 mm pre coated silica gel aluminum plates (60 F254) using mixture of petroleum ether and ethyl acetate. Visualization of the spots on TLC plates was achieved by exposure to UV light. Column chromatography was performed using silica gel (Acme, 100-200mesh). Solvents were dried and purified by distillation prior to use. Solvents for chromatography (Petroleum ether and ethyl acetate) were distilled prior to use. Evaporations were carried out under reduced pressure on Heidolph rotary evaporator. Melting points were obtained using Stuart SMP30 system and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Avance-III 400MHz (400 MHz for ^{1}H, 100 MHz for ^{13}C), in $CDCl_3$ or DMSO-d_6. Chemical shifts have been expressed in parts per million (δ) relative to tetramethylsilane (δ = 0.0) as an internal standard and coupling constants (*J*) in Hertz. Low-resolution mass spectra (LC-MS) were recorded on LC/MS-2020 Shimadzu. IR spectra were recorded as KBr pellets on Jasco FTIR-4200 spectrometer.

General procedure for the synthesis of 4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine (**24**)

To a stirred solution of cyanuric chloride (10.0 g, 0.054 mol) in acetone (100 ml) a solution of morpholine (3.4 g, 0.039 mol) in acetone (100 ml) and triethylamine (3.9 g, 0.039 mol) were added at -20 °C. The mixture was then quenched with H_2O, stirred for a few minutes, filtered, washed with MeOH and dried to obtain the product **24** as white powder. Yield: 8.4 g, 93.0% [34].

General procedure for the synthesis of 4-chloro-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**25**)

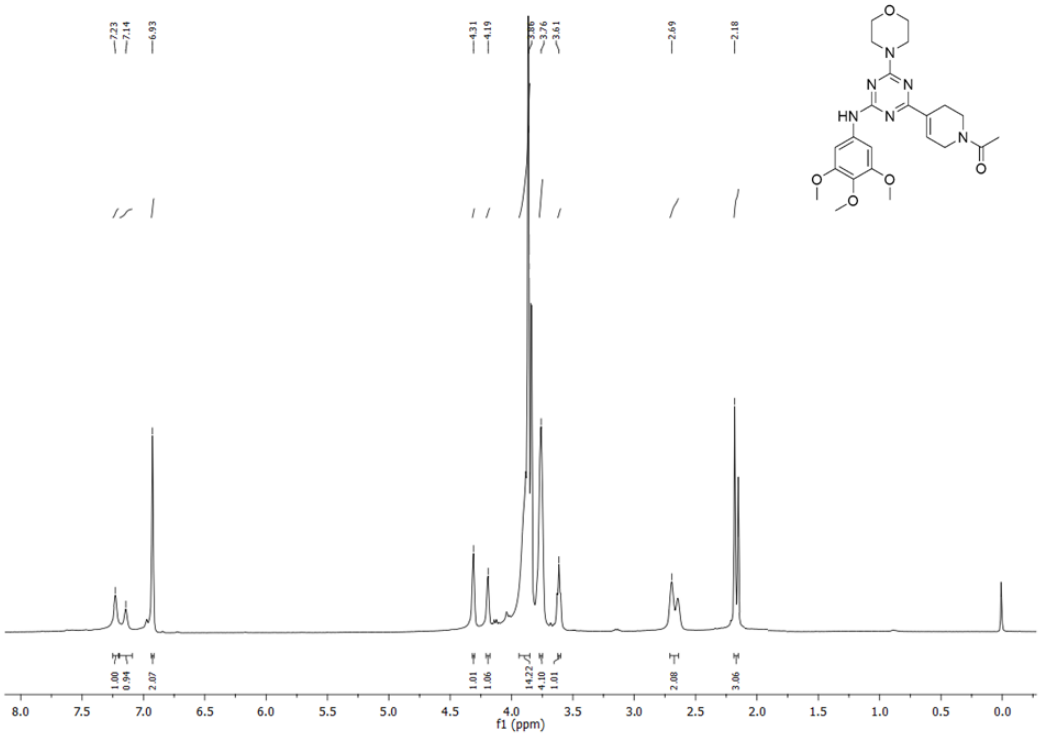
To a stirred solution of 4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine (**24**) (1g, 0.0042 mol) in 1,4-dioxane (10ml) 3,4,5-trimethoxy aniline (0.78g, 0.0042 mol) and DIPEA (1.1 mL, 0.0063 mol) were added at RT for 6h. Reaction was monitored by TLC and water was added to reaction mixture once complete and was followed by extraction with ethyl acetate. Combined organic layers were collected and dried over dry sodium sulfate and concentrated in vacuo to yield the product **25** as pale yellow solid.

General procedure for the synthesis of tert-butyl-4-(4-morpholino-6-(3,4,5-trimethoxy phenyl amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (**26**)

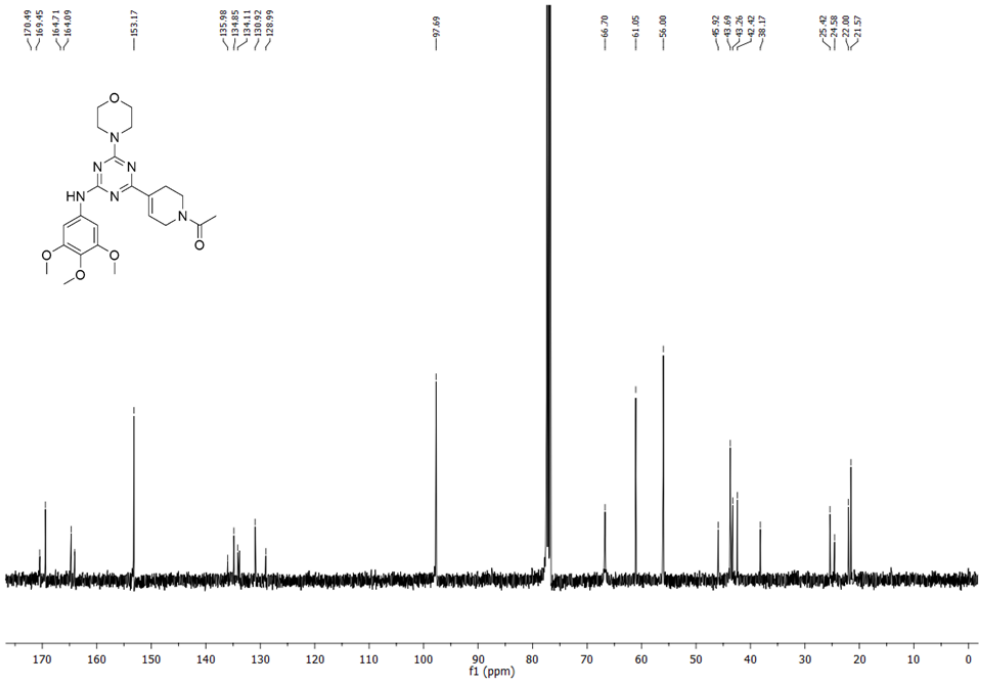
Synthesized tert-butyl-4-(4-morpholino-6-(3,4,5-trimethoxy phenyl amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (**26**) by reported reaction conditions [35]. To a stirred solution of 4-chloro-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**25**) (1g, 0.0026 mol) in 1,4-dioxane:H_2O (3:1mL) *N*-Boc-1,2,5,6-tetrahydropyridine-4-boronic acid pinacol ester (0.81g, 0.0026 mol) and Pd(dppf)Cl_2 (0.095g, 0.00013mol) under inert conditions were added and maintained at reflux for 6h. Reaction was monitored by TLC and water was added to reaction mixture once complete and was followed by extraction with ethyl acetate. Combined organic layers were collected and dried over dry sodium sulphate and concentrated in vacuo to yield **26** as brown solid.

General procedure for the synthesis of 1-(4-(4-morpholino-6-(3,4,5-trimethoxyphenylamino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl) substituted ethanone (**28a-b**)

4-morpholino-6-(1,2,3,6-tetrahydropyridin-4-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**27**) was synthesized from compound **26**, by deprotection with trifluoro acetic acid at 0 °C. To a stirred solution of 4-morpholino-6-(1,2,3,6-tetrahydropyridin-4-yl)-N-(3,4,5-trimethoxy phenyl)-1,3,5-triazin-2-amine (**27**) (0.3g, 0.7mmol) in DCM at 0 °C, various anhydrides (0.1g, 1.05mmol) and triethylamine (0.35g, 3.5mmol) were added. Reaction was monitored by TLC and water was added to reaction mixture once complete and was followed by extraction with ethyl acetate. Combined organic layers were collected and dried over dry sodium sulphate and concentrated invacuo and washed with DEE to yield final product.



^{1}H NMR spectrum of compound **28a**



^{13}C NMR spectrum of compound **28a**

1-(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydro pyridin-1(2H)-yl)ethanone (**28a**)

Brown solid; yield: 78%, 0.25g, m.p. 215-217 °C; IR (KBr, cm^{-1}) 3415, 3045, 1725, 1255, 1195, 1120. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (br, 1H), 7.14 (s, 1H), 6.93 (s, 2H), 4.31 (s, 1H), 4.19 (s, 1H), 3.86 (d, *J* = 7.5 Hz, 14H), 3.76 (s, 4H), 3.61 (s, 1H), 2.69 (d, *J* = 19.5 Hz, 2H), 2.18 (d, *J* = 11.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.49, 169.45, 164.71, 164.09, 153.17, 135.98, 134.85, 134.11, 130.92, 128.99, 97.69, 66.70, 61.05, 56.00, 45.92, 43.69, 43.26, 42.42, 38.17, 25.42, 24.58, 22.00, 21.57. ESI-MS (m/z): calcd. for $C_{23}H_{30}N_6O_5$ 470.23, found 471.31 $[M + H]^+$.

2,2,2-trifluoro-1-(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)ethanone (**28b**)

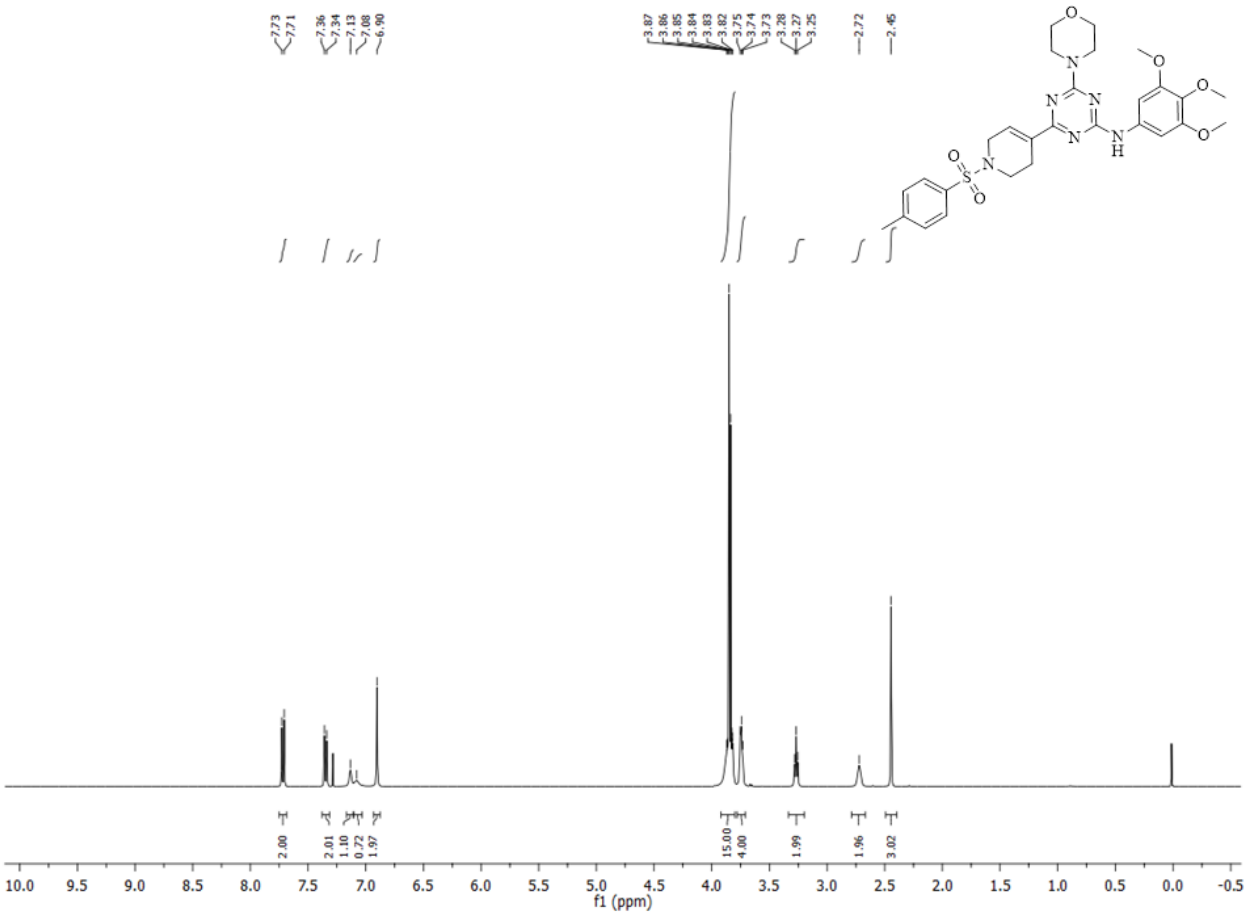
Brown solid; yield: 89%, 0.32g, m.p. 189-190 °C; IR (KBr, cm^{-1}) 3445, 3043, 1276, 1180, 1123, 1075. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (br, 1H), 7.22 (s, 1H), 6.85 (s, 2H), 4.12 (s, 2H), 3.91 (m, 14H), 3.81 (m, 5H), 2.67 (d, *J* = 19.72 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.92, 169.83, 165.41, 154.37, 153.72, 137.27, 135.74, 132.72, 130.43, 119.4, 98.32, 67.20, 61.85, 56.93, 46.27, 43.87, 42.71, 38.94, 26.47. ESI-MS (m/z): calcd. for $C_{23}H_{27}F_3N_6O_5$ 524.20, found 525.27 $[M + H]^+$.

General procedure for the synthesis of 4-(1-(substitutedsulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29a-l**)

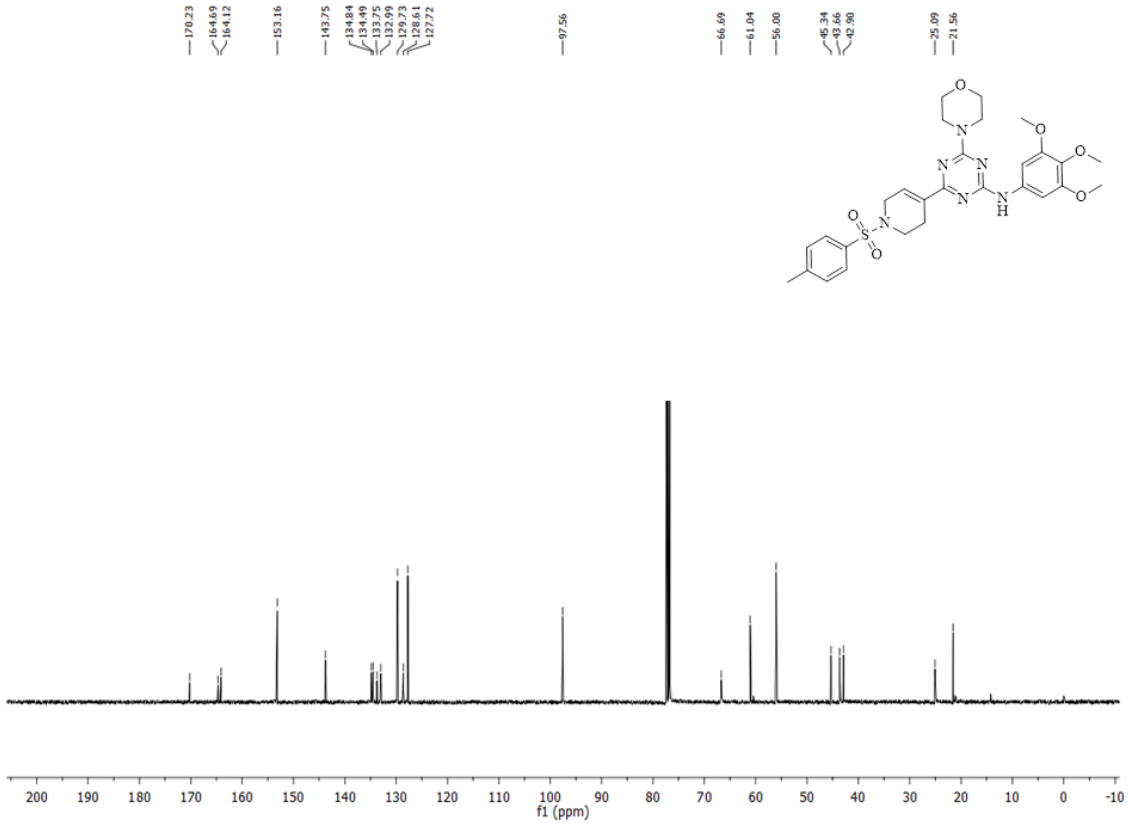
To a stirred solution of 4-morpholino-6-(1,2,3,6-tetrahydropyridin-4-yl)-N-(3,4,5-trimethoxy phenyl)-1,3,5-triazin-2-amine (**27**) (0.3g, 0.7mmol) in DCM, sulfonyl chloride (0.7mmol) and triethylamine (0.21g, 2.1mmol) were added at 0 °C-RT. Reaction was monitored by TLC and water was added to reaction mixture once complete and was followed by extraction with ethyl acetate. Combined organic layers were collected and dried over dry sodium sulphate. Concentrated the organic layers and purified by column chromatography with 30% ethyl acetate in petroleum ether to yield the title compounds.

4-(1-(methylsulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29a**)

Brown solid; yield: 95%, 0.33g, m.p. 211-213 °C; IR (KBr, cm^{-1}) 3425, 3040, 1270, 1185, 1123, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (br, 1H), 7.17 (s, 1H), 6.95 (s, 1H), 4.32 (s, 1H), 4.21 (s, 1H), 3.87 (m, 14H), 3.78 (s, 5H), 3.63 (s, 1H), 2.69 (d, *J* = 19.7 Hz, 2H), 2.95 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.46, 169.47, 164.72, 153.19, 135.99, 135.29 – 135.0 (m), 134.50 (d, *J* = 75.3 Hz), 133.81, 130.93, 129.9, 97.70, 77.40, 77.16, 76.76, 66.72, 61.13, 56.14, 45.97, 43.71, 43.29, 42.45, 38.19, 25.45. ESI-MS (m/z): calcd. for $C_{22}H_{30}N_6O_6S$ 506.19, found 507.22 $[M + H]^+$.



^{1}H NMR spectrum of compound **29b**



^{13}C NMR spectrum of compound **29b**

4-morpholino-6-(1-tosyl-1,2,3,6-tetrahydropyridin-4-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29b**)

White solid; yield: 79%, 0.32g, m.p. 216-218 °C; IR (KBr, cm^{-1}) 3430, 3035, 1275, 1180, 1125, 1047. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.72 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.13 (br, 1H), 7.08 (s, 1H), 6.90 (s, 2H), 3.87-3.82 (m, 15H), 3.75 – 3.72 (m, 4H), 3.27 (t, *J* = 5.7 Hz, 2H), 2.72 (s, 2H), 2.45 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.23, 164.69, 164.12, 153.16, 143.75, 134.84, 134.49, 133.75, 132.99, 129.73, 128.61, 127.72, 97.56, 66.69, 61.04, 56.00, 45.34, 43.66, 42.90, 25.09, 21.56. ESI-MS (m/z): calcd. for $C_{28}H_{34}N_6O_6S$ 582.23, found 583.27 $[M + H]^+$.

4-(1-((4-methoxyphenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29c**)

Brown solid; yield: 87%, 0.36g, m.p. 209-211 °C; IR (KBr, cm^{-1}) 3437, 3046, 1273, 1184, 1123, 1048. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.75 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H),

7.18 (br, 1H), 7.15(s, 1H), 6.94 (s, 2H), 3.85 (m, 15H), 3.78 – 3.74 (m, 4H), 3.47 (s, 3H).3.29 (t, *J* = 5.7 Hz, 2H), 2.75 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.37, 164.72, 164.16, 153.19, 143.79, 134.89, 134.52, 133.79, 133.10, 129.76, 128.65, 127.75, 97.59, 77.41, 77.12, 76.79, 66.73, 61.13, 56.12, 45.37, 43.69, 42.96, 25.19. ESI-MS (m/z): calcd. for $C_{28}H_{34}N_6O_7S$ 598.22, found 599.27 $[M + H]^+$.

4-(1-((4-fluorophenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29d**)

Brown solid; yield: 86%, 0.35g, m.p. 219-220 °C; IR (KBr, cm^{-1}) 3420, 3043, 1271, 1182, 1122, 1095, 1048. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.78 (d, *J* = 8.31 Hz, 2H), 7.39 (d, *J* = 8.12 Hz, 2H), 7.21 (br, 1H), 7.17 (s, 1H), 6.97 (s, 2H), 3.88 (m, 15H), 3.79 – 3.76 (m, 4H), 3.28 (t, *J* = 5.7 Hz, 2H), 2.76 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.36, 164.71, 164.18, 153.21, 143.78, 134.91, 134.55, 133.81, 133.12, 129.78, 128.67, 127.76, 97.61, 77.42, 77.13, 76.81, 66.75, 61.15, 45.39, 43.71, 42.98, 25.20. ESI-MS (m/z): calcd. for $C_{27}H_{31}FN_6O_6S$ 586.20, found 587.24 $[M + H]^+$.

4-(1-((4-bromophenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29e**)

White solid; yield: 89%, 0.40g, m.p. 194-196 °C; IR (KBr, cm^{-1}) 3445, 3043, 1276, 1180, 1123, 1042, 575. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.75 (d, *J* = 8.29 Hz, 2H), 7.37 (d, *J* = 8.11 Hz, 2H), 7.20 (br, 1H), 7.15 (s, 1H), 6.95 (s, 2H), 3.85 (m, 15H), 3.77 – 3.75 (m, 4H), 3.25 (t, *J* = 5.7 Hz, 2H), 2.72 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.32, 164.69, 164.16, 153.19, 143.75, 134.89, 134.51, 133.79, 133.14, 129.75, 128.65, 127.75, 97.58, 77.39, 77.10, 76.77, 66.72, 61.12, 45.36, 43.67, 42.91, 25.21. ESI-MS (m/z): calcd. for $C_{27}H_{31}BrN_6O_6S$ 646.12, found 647.18 $[M + H]^+$.

4-morpholino-6-(1-((4-nitrophenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-N-(3,4,5-tri methoxyphenyl)-1,3,5-triazin-2-amine (**29f**)

Brown solid; yield: 95%, 0.40g, m.p. 214-216 °C; IR (KBr, cm^{-1}) 3425, 3040, 1545, 1350, 1273, 1182, 1123, 1042. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 8.12 (d, *J* = 8.47 Hz, 2H), 7.89 (d, *J* = 8.32 Hz, 2H), 7.31 (br, 1H), 7.23 (s, 1H), 7.06 (s, 2H), 3.94 (m, 15H), 3.81–3.79 (m, 4H), 3.29 (t,

J = 5.79 Hz, 2H), 2.31 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.12, 165.2, 164.82, 153.79, 144.52, 135.91, 134.94, 133.87, 133.76, 130.65, 129.10, 128.23, 98.68, 78.43, 77.9, 78.8, 67.32, 61.94, 46.52, 44.76, 43.95, 27.63. ESI-MS (m/z): calcd. for $C_{27}H_{31}N_7O_8S$, 613.20, found 614.28 $[M + H]^+$.

4-morpholino-6-(1-((4-(trifluoromethyl)phenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29g**)

White solid; yield: 91%, 0.40g, m.p. 212-214 °C; IR (KBr, cm^{-1}) 3445, 3043, 1276, 1180, 1123, 1056, 1030. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.81 (d, *J* = 8.32 Hz, 2H), 7.42 (d, *J* = 8.13 Hz, 2H), 7.23 (br, 1H), 7.19 (s, 1H), 6.98 (s, 2H), 3.89 (m, 15H), 3.81 –3.77 (m, 4H), 3.29 (t, *J* = 5.7 Hz, 2H), 2.79 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.39, 164.72, 164.19, 153.23, 143.79, 134.93, 134.57, 133.82, 133.14, 129.8, 128.69, 127.78, 97.63, 77.44, 77.15, 76.83, 66.76, 61.18, 45.42, 43.75, 43.81, 25.29. ESI-MS (m/z): calcd. for $C_{28}H_{31}F_3N_6O_6S$ 636.20, found 637.24 $[M + H]^+$.

4-(1-((5-bromothiophen-2-yl)sulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29h**)

White solid; yield: 92%, 0.42g, m.p. 185-186 °C; IR (KBr, cm^{-1}) 3442, 3041, 1274, 1180, 1124, 1046, 552. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.69 (d, *J* = 8.19 Hz, 2H), 7.34 (d, *J* = 8.06 Hz, 2H), 6.97 (br, 1H), 6.85 (s, 1H), 6.69 (s, 2H), 3.78 (m, 15H), 3.69 – 3.61 (m, 4H), 3.19 (t, *J* = 5.6 Hz, 2H), 2.64 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.06, 164.12, 163.95, 152.93, 142.9, 133.9, 132.61, 131.7, 130.74, 129.16, 127.85, 126.47, 97.18, 77.25, 77.12, 76.14, 66.72, 60.72, 44.16, 43.12, 42.15, 25.07. ESI-MS (m/z): calcd. for $C_{25}H_{29}BrN_6O_6S_2$ 652.08, found 653.17 $[M + H]^+$.

1-(4-((4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)sulfonyl)phenyl)ethanone (**29i**)

Pale yellow solid; yield: 85%, 0.36g, m.p. 210-211 °C; IR (KBr, cm^{-1}) 3445, 3043, 1734, 1276, 1195, 1120, 1040. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.69 (d, *J* = 8.21 Hz, 2H), 7.24 (d, *J* = 8.03 Hz, 2H), 7.06 (br, 1H), 6.95 (s, 1H), 6.87 (s, 2H), 3.79 (m, 15H), 3.71 – 3.69 (m, 4H), 3.19 (t, *J* = 5.61 Hz, 2H), 2.68 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.16, 165.28, 164.15, 154.6,

144.25, 135.24, 134.9, 134.07, 133.69, 130.15, 129.24, 128.15, 98.38, 77.92, 77.34, 76.58, 67.84, 62.71, 46.15, 44.73, 43.54, 26.47. ESI-MS (m/z): calcd. for $C_{29}H_{34}N_6O_7S$ 610.22, found 611.28 $[M + H]^+$.

4-(1-((4-chlorophenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29j**)

Brown solid; yield: 87%, 0.36g, m.p. 161-162 °C; IR (KBr, cm^{-1}) 3438, 3040, 1275, 1184, 1123, 1045, 760. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.72 (m, 2H), 7.60 – 7.48 (m, 2H), 7.13 (br, 1H), 7.05 (s, 1H), 6.90 (s, 2H), 3.85 (m, 15H), 3.78 – 3.71 (m, 4H), 3.30 (t, *J* = 5.7 Hz, 2H), 2.72 (s, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 170.07, 164.67, 164.06, 153.18, 139.48, 135.03, 134.46, 133.83, 129.46, 129.02, 128.27, 97.64, 66.67, 61.05, 56.01, 45.28, 43.67, 42.86, 24.98. ESI-MS (m/z): calcd. for $C_{27}H_{31}ClN_6O_6$, 602.17, found 603.21 $[M + H]^+$.

4-(1-((4-(tert-butyl)phenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-6-morpholino-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29k**)

Brown solid; yield: 92%, 0.40g, m.p. 212-214 °C; IR (KBr, cm^{-1}) 3445, 3040, 1275, 1182, 1121, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.14 (d, *J* = 8.06 Hz, 2H), 6.97 (d, *J* = 7.91 Hz, 2H), 6.95 (br, 1H), 6.91 (s, 1H), 6.83 (s, 2H), 3.78 (m, 15H), 3.64 – 3.60 (m, 4H), 3.14 (t, *J* = 5.27 Hz, 2H), 2.69 (s, 2H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.56, 163.95, 163.17, 151.46, 142.37, 133.24, 132.83, 131.89, 130.37, 128.65, 127.49, 126.32, 96.81, 74.69, 65.29, 60.84, 55.30, 44.72, 42.93, 41.70, 30.65, 25.09. ESI-MS (m/z): calcd. for $C_{31}H_{40}N_6O_6S$ 624.27, found 625.35 $[M + H]^+$.

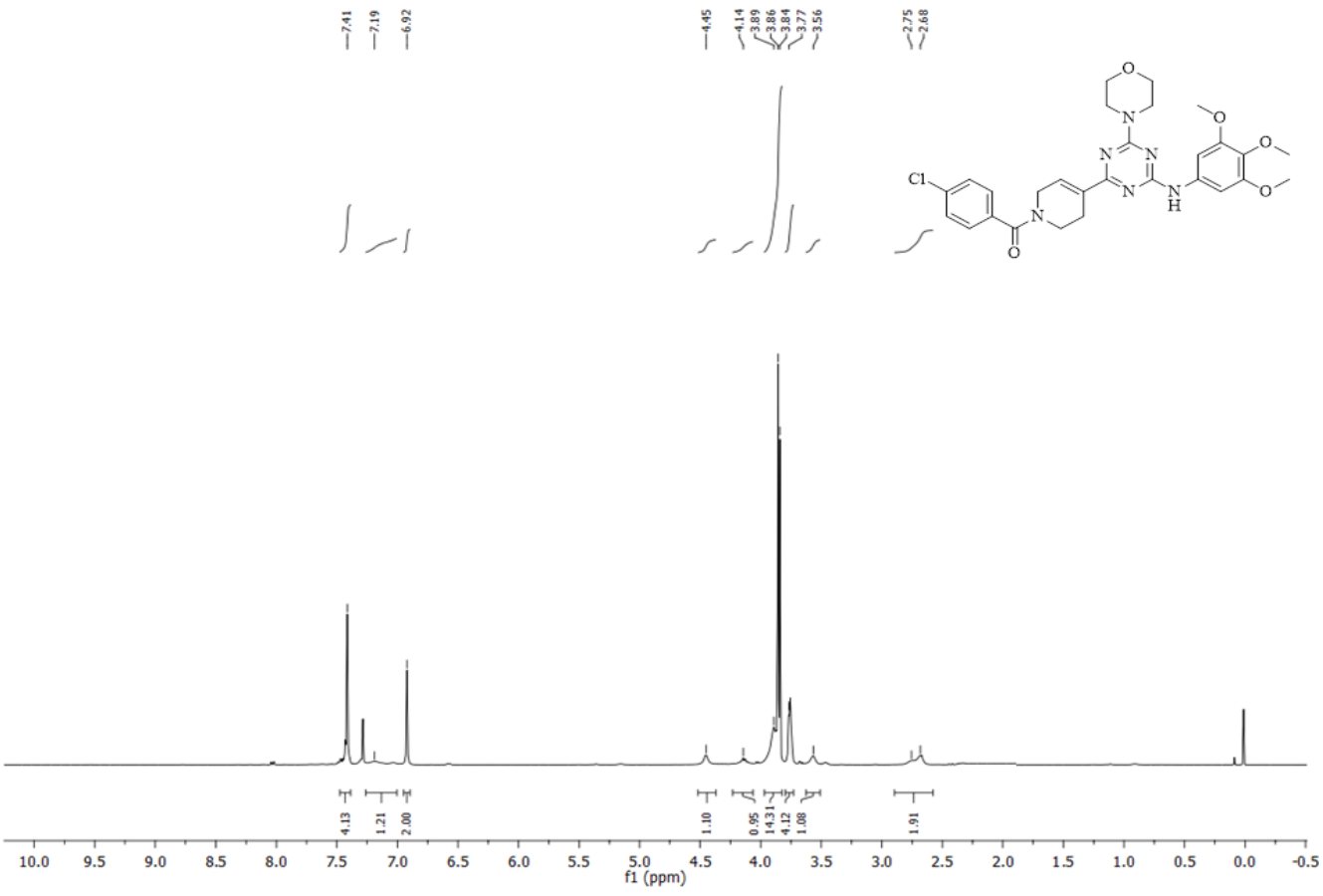
4-morpholino-6-(1-((2-nitrophenyl)sulfonyl)-1,2,3,6-tetrahydropyridin-4-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine (**29l**)

Pale yellow solid; yield: 76%, 0.32g, m.p. 206-208 °C; IR (KBr, cm^{-1}) 3425, 3040, 1530, 1345, 1275, 1185, 1120, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 8.45 (d, *J* = 8.61 Hz, 1H), 8.27 (d, *J* = 8.46 Hz, 1H), 8.11 (m, 1H), 7.93 (m, 1H), 7.25 (br, 1H), 7.14 (s, 1 H), 6.95(s, 2H), 3.94 (m, 15H), 3.85–3.71 (m, 4H), 3.47 (t, *J* = 5.82 Hz, 2H), 2.38 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.23, 165.31, 164.89, 154.27, 146.59, 144.93, 136.48, 135.26, 134.17, 133.98, 131.29, 130.12,

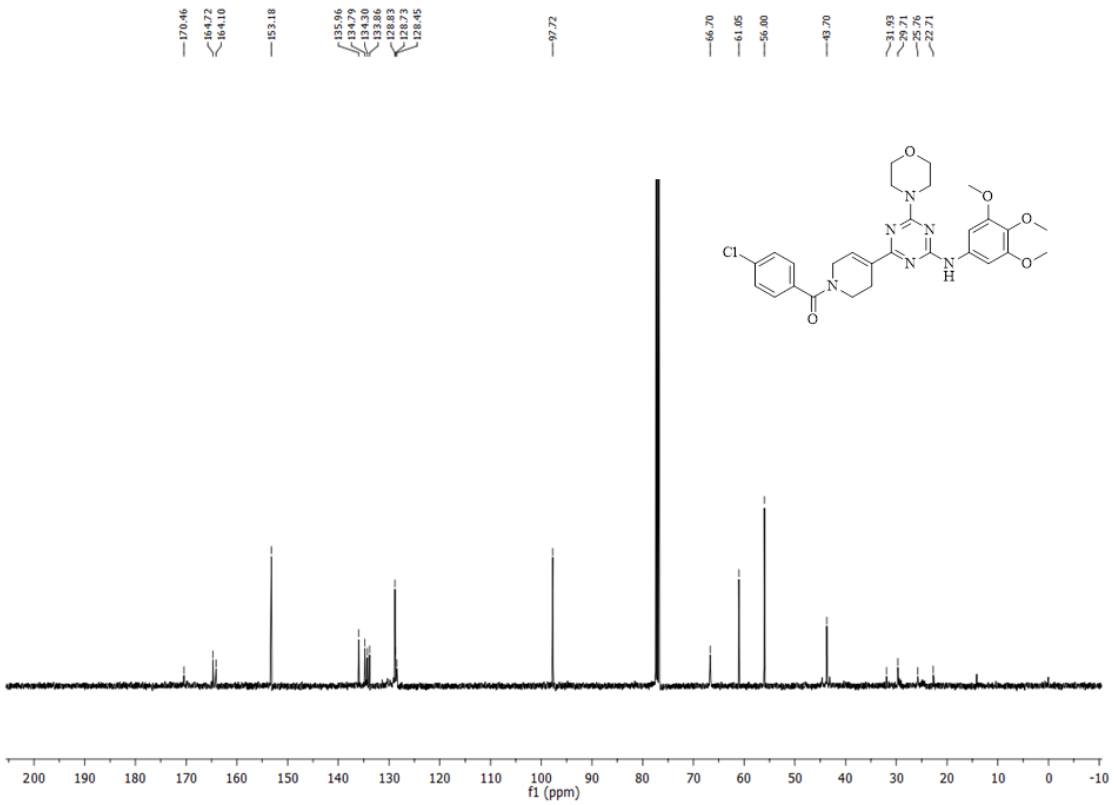
129.83, 127.40, 123.76, 99.71, 67.91, 56.35, 62.74, 47.32, 45.61, 44.27, 29.50. ESI-MS (m/z): calcd. for $C_{27}H_{31}N_7O_8S$, 613.20, found 614.28 $[M + H]^+$.

General procedure for the synthesis of 1-(4-(4-morpholino-6-(3,4,5-trimethoxyphenylamino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)substitutedmethanone (**30a-l**)

To a stirred solution of 4-morpholino-6-(1,2,3,6-tetrahydropyridin-4-yl)-N-(3,4,5-trimethoxy phenyl)-1,3,5-triazin-2-amine (**27**) (0.3g, 0.7mmol) in DCM, acid (0.7mmol), triethylamine (0.21g, 2.1mmol), HOBt (21mg, 0.14mmol), EDC.HCl (0.26g, 1.4mmol) were added at RT. Reaction was monitored by TLC and water was added to reaction mixture once complete and was followed by extraction with ethyl acetate. Combined organic layers were collected and dried over dry sodium sulfate. Concentrated the organic layers and purified by column chromatography with 45% ethyl acetate in petroleum ether.



^{1}H NMR spectrum of compound **30a**



^{13}C NMR spectrum of compound **30a**

(4-chlorophenyl)(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl) methanone (**30a**)

Pale yellow solid; yield: 78%, 0.30g, m.p. 208-210 °C; IR (KBr, cm^{-1}) 3440, 3043, 1670, 1276, 1180, 1120, 758. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 4H), 7.19 (br, 1H), 7.13 (s, 1H), 6.92 (s, 2H), 4.45 (s, 1H), 4.14 (s, 1H), 3.89-3.84 (m, 14H), 3.77 (s, 4H), 3.56 (s, 1H), 2.72 (d, *J* = 28.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.46, 164.72, 164.10, 153.18, 135.96, 134.79, 134.30, 133.86, 129.16, 128.45, 128.45, 128.13, 97.72, 66.70, 61.05, 56.00, 43.70, 31.93, 29.71, 25.76, 22.71. ESI-MS (m/z): calcd. for $C_{28}H_{31}ClN_6O_5$, 566.20, found 567.29 $[M + H]^+$.

(4-methoxyphenyl)(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)methanone (**30b**)

White solid; yield: 73%, 0.28g, m.p. 206-208 °C; IR (KBr, cm^{-1}) 3440, 3040, 1679, 1279, 1180, 1125, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.91 (d, *J* = 8.41 Hz, 2H), 7.42 (d, *J* = 8.32 Hz,

2H), 7.23 (br, 1H), 7.20(s, 1H), 6.98 (s, 2H), 3.92-3.85 (m, 15H), 3.81 – 3.79 (m, 4H), 3.52 (s, 3H).3.34 (t, *J* = 5.72 Hz, 2H), 2.81 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.41, 167.53, 165.32, 164.92, 154.73, 144.65, 136.69, 135.90, 134.79, 133.17, 129.76, 128.65, 127.75, 98.59, 76.79, 66.73, 61.13, 56.12, 45.37, 43.65, 42.91, 27.36. ESI-MS (m/z): calcd. for $C_{29}H_{34}N_6O_6$ 562.25, found 562.62 $[M + H]^+$.

2-mercapto-1-(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)ethanone (**30c**)

White solid; yield: 78%, 0.27g, m.p. 217-218 °C; IR (KBr, cm^{-1}) 3430, 3040, 2575, 1680, 1275, 1185, 1123. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (br, 1H), 7.06 (s, 1H), 6.85 (s, 2H), 3.91-3.87 (m, 2H), 3.85-3.83 (m, 14H), 3.81-3.78 (m, 5H), 3.57(s, 2H), 2.67 (t, *J* = 19.52 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.32, 169.41, 165.73, 157.93, 153.68, 135.27, 132.59, 130.43, 121.79, 98.50, 67.20, 61.85, 56.93, 46.27, 43.87, 42.71, 30.94, 28.47. ESI-MS (m/z): calcd. for $C_{23}H_{30}N_6O_5S$ 502.20, found 502.59 $[M + H]^+$.

3-(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydro pyridin-1(2H)-yl)-3-oxopropanenitrile (**30d**)

Pale yellow solid; yield: 76%, 0.26g, m.p. 205-206 °C; IR (KBr, cm^{-1}) 3435, 3040, 2250, 1685, 1270, 1185, 1120. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (br, 1H), 7.18 (s, 1H), 6.91 (s, 2H), 3.94-3.91 (m, 2H), 3.86-3.79 (m, 15H), 3.81-3.78 (m, 4H), 3.61(s, 2H), 2.70 (t, *J* = 19.71 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.64, 169.52, 165.81, 158.43, 153.90, 137.50, 135.74, 132.65, 131.20., 121.83, 99.42, 67.79, 62.35, 57.13, 46.86, 44.27, 42.71, 31.69, 29.38. ESI-MS (m/z): calcd. for $C_{24}H_{29}N_7O_5$ 495.22, found 495.53 $[M + H]^+$.

furan-2-yl(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)methanone (**30e**)

Pale yellow solid; yield: 84%, 0.30g, m.p. 179-181 °C; IR (KBr, cm^{-1}) 3440, 3045, 1680, 1270, 1185, 1120. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 3.92 Hz, 1H), 7.19 (d, *J* = 3.64 Hz, 1H), 7.06 (m, 3H), 6.95 (s, 2H), 4.14 (t, *J* = 5.37 Hz, 2H), 3.89-3.80 (m, 15H), 3.68 (s, 4H), 2.72 (d, *J* = 27.46 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.46, 165.21, 164.76, 162.50, 153.69, 138.90, 136.29, 135.42, 133.65, 129.54, 128.37, 124.60, 121.92, 98.42, 72.18, 67.15, 65.83,

63.49, 57.29, 43.70, 31.93, 29.71. ESI-MS (m/z): calcd. for $C_{26}H_{30}N_6O_6$, 522.22, found 522.55 $[M + H]^+$.

(2-chloropyridin-3-yl)(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)methanone (**30f**)

Pale yellow solid; yield: 72%, 0.28g, m.p. 217-218 °C; IR (KBr, cm^{-1}) 3440, 3040, 1680, 1275, 1180, 1120, 755. ^1H NMR (400 MHz, $CDCl_3$) δ ppm, 8.31 (d, *J* = 8.54 Hz, 1H), 7.42 (d, *J* = 7.63 Hz, 1H), 7.20 (t, *J* = 5.63 Hz, 1H), 7.13 (br, 1H), 7.10(s, 1H), 6.95 (s, 2H), 3.94-3.89 (m, 15H), 3.81 – 3.79 (m, 4H), 3.54 (t, *J* = 5.72 Hz, 2H), 2.81 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.81, 167.39, 165.21, 164.73, 155.73, 145.15, 142.46, 137.39, 136.20, 134.83, 133.67, 130.25, 128.65, 127.75, 99.24, 76.49, 66.72, 61.43, 56.20, 45.39, 43.27, 42.63, 28.65. ESI-MS (m/z): calcd. for $C_{29}H_{30}ClN_7O_5$ 567.00, found 568.02 $[M + H]^+$.

(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)(pyrazin-2-yl)methanone (**30g**)

Pale yellow solid; yield: 85%, 0.31g, m.p. 197-199 °C; IR (KBr, cm^{-1}) 3443, 3045, 1685, 1270, 1185, 1115. ^1H NMR (400 MHz, $CDCl_3$) δ ppm, 8.96 (s, 1H), 8.29 (d, *J* = 8.25 Hz, 1H), 8.14 (d, *J* = 7.83 Hz, 1H), 7.16 (br, 1H), 7.11(s, 1H), 7.05 (s, 2H), 3.96-3.90 (m, 15H), 3.84 – 3.79 (m, 4H), 3.56 (t, *J* = 5.75 Hz, 2H), 2.85 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.85, 167.59, 165.47, 164.90, 156.13, 145.85, 143.26, 138.52, 137.10, 135.76, 134.62, 131.25, 129.35, 127.90, 99.68, 76.50, 67.42, 62.55, 57.90, 46.27, 44.92, 43.5, 29.15. ESI-MS (m/z): calcd. for $C_{26}H_{30}N_8O_5$ 534.23, found 534.57 $[M + H]^+$.

2-(1H-indol-3-yl)-1-(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)ethanone (**30h**)

White solid; yield: 85%, 0.32g, m.p. 106-107 °C; IR (KBr, cm^{-1}) 3441, 3042, 1690, 1270, 1185, 1120. ^1H NMR (400 MHz, $CDCl_3$) δ 10.94 (s, 1H), 8.39 (d, *J* = 8.62 Hz, 1H), 7.42 (d, *J* = 7.63 Hz, 1H), 7.34 (d, *J* = 8.06 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 1H), 7.19 (d, *J* = 3.64 Hz, 1H), 7.08 (br, 1H), 7.02 (s, 1H), 6.90 (s, 2H), 3.88-3.82 (m, 2H), 3.81 (m, 14H), 3.81 (m, 5H), 3.47(s, 2H), 2.58 (t, *J* = 19.43 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.12, 169.24, 165.43, 157.20, 152.18, 135.27, 133.72, 132.59, 131.43, 129.63, 127.5, 125.40, 123.72, 121.79, 120.65, 119.43,

118.60, 116.34, 98.27 , 67.25, 61.52, 57.13, 46.21, 43.82, 42.68, 30.65, 28.30. ESI-MS (m/z): calcd. for $C_{31}H_{35}N_7O_5$ 585.27, found 585.65 $[M + H]^+$.

(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)(pyridin-4-yl)methanone (**30i**)

White solid; yield: 90%, 0.33g, m.p. 223-225 °C; IR (KBr, cm^{-1}) 3441, 3044, 1685, 1275, 1185, 1120. ^1H NMR (400 MHz, $CDCl_3$) δ ppm, 8.72 (d, *J* = 8.24 Hz, 2H), 7.46 (d, *J* = 7.65 Hz, 2H), 7.16 (br, 1H), 7.09(s, 1H), 6.98 (s, 2H), 3.95-3.90 (m, 15H), 3.82–3.78 (m, 4H), 3.56 (t, *J* = 5.74 Hz, 2H), 2.83 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.85, 167.42, 165.35, 164.81, 155.70, 147.5, 145.2, 142.46, 137.29, 136.20, 130.25, 128.65, 127.75, 99.24, 76.49, 66.72, 61.43, 56.20, 45.39, 43.27, 42.63, 28.65. ESI-MS (m/z): calcd. for $C_{27}H_{31}N_7O_5$ 533.24, found 533.58 $[M + H]^+$.

(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)(5-nitrofuran-2-yl) methanone (**30j**)

Yellow solid; yield: 81%, 0.32g, m.p. 193-195 °C; IR (KBr, cm^{-1}) 3445, 3040, 1685, 1530, 1325, 1270, 1180, 1125. ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 3.8 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.18 (s, 1H), 6.93 (s, 2H), 4.53 (d, *J* = 80.7 Hz, 2H), 3.85 (m, 15H), 3.77 (s, 4H), 2.82 (d, *J* = 44.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.23, 164.69, 164.11, 153.19, 134.77, 133.86, 129.31, 118.24 (d, *J* = 37.1 Hz), 111.77, 97.69, 77.38, 77.06, 76.74, 66.67, 61.05, 56.02, 43.71, 25.79, 24.86. ESI-MS (m/z): calcd. for $C_{26}H_{29}N_7O_8$ 567.21, found 568.25 $[M + H]^+$.

(4-bromophenyl)(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)methanone (**30k)**

White solid; yield: 71%, 0.30g, m.p. 218-220 °C; IR (KBr, cm^{-1}) 3435, 3035, 1685, 1270, 1180, 1120, 565. ^1H NMR (400 MHz, $CDCl_3$) δ 7.41-7.39 (s, 4H), 7.16 (br, 1H), 7.08(s, 1H), 6.94 (s, 2H), 4.14 (t, *J* = 10.1 Hz, 2H), 3.86-3.80 (m, 15H), 3.75 (s, 4H), 2.75 (d, *J* = 28.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.61, 164.75, 164.12, 153.18, 135.96, 134.79, 134.30, 133.86, 129.16, 128.45, 128.45, 128.14, 97.74, 77.48, 77.16, 76.75, 66.72, 61.15, 56.00, 43.70, 31.93, 29.73, 25.76, 22.75. ESI-MS (m/z): calcd. for $C_{28}H_{31}BrN_6O_5$, 610.15, found 611.49 $[M + H]^+$.

(4-bromo-2-hydroxyphenyl)(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)methanone (**30l**)

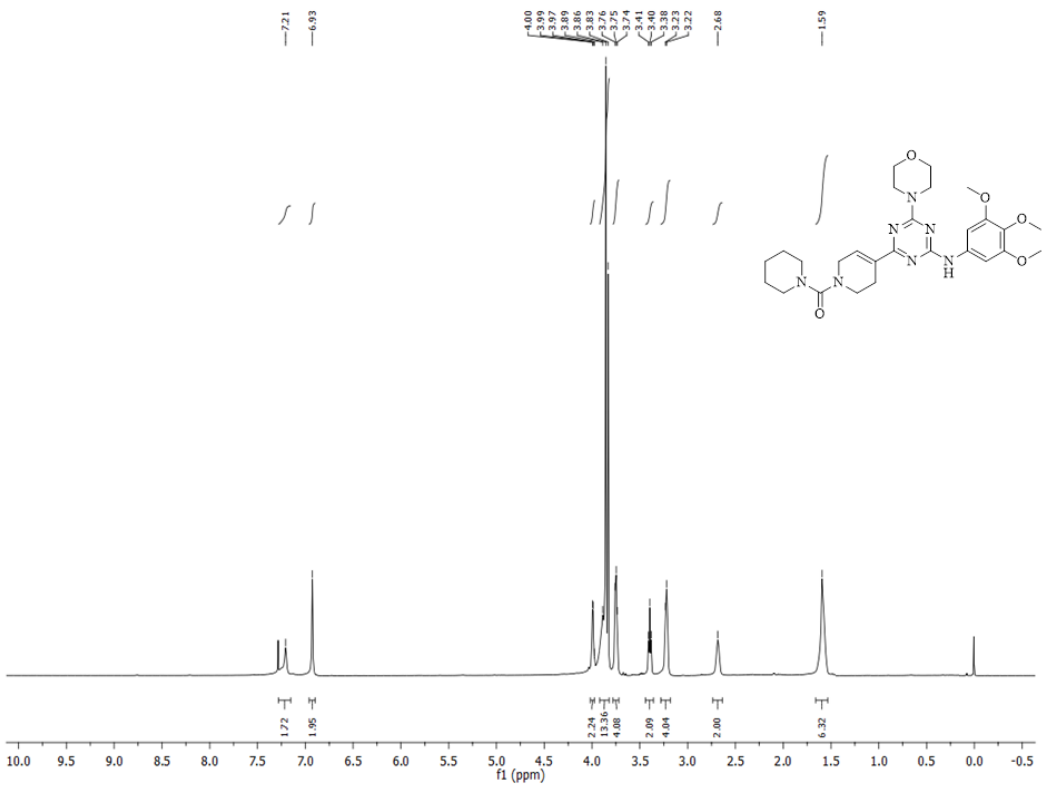
White solid; yield: 89%, 0.39g, m.p. 150-151 °C; IR (KBr, cm^{-1}) 3440, 3290, 3040, 1680, 1275, 1180, 1120, 570. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.25 (s, 1H), 7.45 (s, 1H), 7.39 (d, *J* = 3.8 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.18 (br, 1H), 7.11(s, 1H), 6.97 (s, 2H), 4.15 (t, *J* = 10.1 Hz, 2H), 3.87-3.82 (m, 15H), 3.78 (s, 4H), 2.76 (d, *J* = 28.75 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.42, 164.91, 164.25, 161.45, 153.28, 136.18, 134.82, 134.75, 133.91, 129.20, 128.65, 128.3, 128.21, 97.82, 77.54, 77.29, 76.83, 66.90, 61.25, 56.40, 43.82, 31.96, 29.78, 25.80, 22.74. ESI-MS (m/z): calcd. for $C_{28}H_{31}BrN_6O_6$, 626.15, found 627.49 $[M + H]^+$.

General procedure for the synthesis of N-substituted-4-(4-morpholino-6-(3,4,5-trimethoxyphenyl amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridine-1(2H)-carboxamide **(31a-k)**

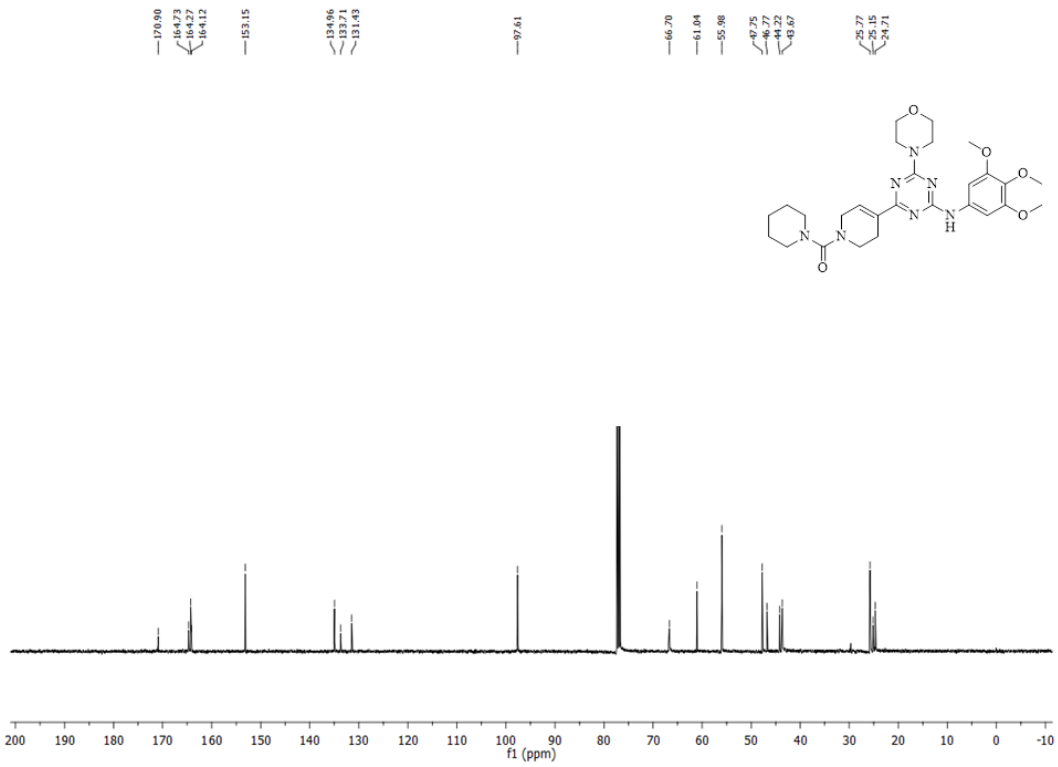
To a stirred solution of 4-morpholino-6-(1,2,3,6-tetrahydropyridin-4-yl)-*N*-(3,4,5-trimethoxy phenyl)-1,3,5-triazin-2-amine (**27**) (0.3g, 0.7mmol) in DCM, corresponding amines (0.7mmol), triethylamine (0.35g, 3.5mmol), triphosgene (0.2g, 0.7mmol) were added at 0 °C. Reaction was monitored by TLC and water was added to reaction mixture once complete and was followed by extraction with ethyl acetate. Combined organic layers were collected and dried over dry sodium sulfate. Concentrated the organic layers and purified by column chromatography with 50% ethyl acetate in petroleum ether to yield the products **31a-k**.

(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)(piperidin-1-yl) methanone (**31a**)

White solid; yield: 82%, 0.30g, m.p. 169-170 °C; IR (KBr, cm^{-1}) 3438, 3042, 1683, 1274, 1182, 1125. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 2H), 6.93 (s, 2H), 3.99 (t, *J* = 4.4 Hz, 2H), 3.98-3.86 (m, 13H), 3.78 – 3.72 (m, 4H), 3.40 (t, *J* = 5.5 Hz, 2H), 3.23 (d, *J* = 4.9 Hz, 4H), 2.68 (s, 2H), 1.59 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.90, 164.73, 164.20 (d, *J* = 15.3 Hz), 153.15, 134.96, 133.71, 131.43, 97.61, 77.39, 77.07, 76.75, 66.70, 61.04, 55.98, 47.75, 46.77, 44.22, 43.67, 25.77, 25.15, 24.71. ESI-MS (m/z): calcd. for $C_{27}H_{37}N_7O_5$, 539.29, found 540.33 $[M + H]^+$.



^{1}H NMR spectrum of Compound **31a**



^{13}C NMR spectrum of Compound **31a**

(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)(pyrrolidin-1-yl) methanone (**31b**)

White solid; yield: 80%, 0.29g, m.p. 194-195 °C; IR (KBr, cm^{-1}) 3432, 3043, 1685, 1270, 1185, 1122. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (br, 1H), 7.16 (s, 1H), 6.93 (s, 2H), 4.02 (d, *J* = 3.0 Hz, 2H), 3.86 (m, 13H), 3.79 – 3.71 (m, 4H), 3.42 (dt, *J* = 13.1, 6.0 Hz, 5H), 2.68 (s, 2H), 1.85 (t, *J* = 6.5 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.92, 164.75, 164.15, 162.74, 153.15, 134.98 (d, *J* = 4.3 Hz), 133.70, 131.37, 97.59, 77.38, 77.06, 76.75, 66.71, 61.04, 55.99, 48.27, 46.20, 43.67, 43.28, 25.58. ESI-MS (m/z): calcd. for $C_{26}H_{35}N_7O_5$ 525.27, found 526.30 $[M + H]^+$.

morpholino(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)methanone (**31c**)

Brown solid; yield: 79%, 0.29g, m.p. 176-178 °C; IR (KBr, cm^{-1}) 3435, 3040, 1685, 1273, 1183, 1122. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (br, 1H), 7.15(s, 1H), 6.98 (s, 2H), 4.02 (d, *J* = 3.5 Hz, 4H), 3.92-3.86 (m, 15H), 3.79 – 3.71 (m, 8H), 3.42 (dt, *J* = 13.4, 6.2 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.88, 168.69, 165.25, 163.34, 154.6, 134.98 (d, *J* = 4.5 Hz), 133.75, 132.46, 125.6, 98.72, 76.24, 74.89, 66.71, 61.04, 55.99, 48.27, 46.20, 44.36, 43.58, 25.68. ESI-MS (m/z): calcd. for $C_{26}H_{35}N_7O_6$ 541.26, found 541.60 $[M + H]^+$.

4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-N-(pyridin-4-yl)-5,6-dihydropyridine-1(2H)-carboxamide (**31d**)

Brown solid; yield: 78%, 0.29g, m.p. 102-103 °C; IR (KBr, cm^{-1}) 3440, 3045, 1685, 1275, 1185, 1120. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 8.52 (d, *J* = 8.21 Hz, 2H), 7.35 (d, *J* = 7.62 Hz, 2H), 7.18 (br, 1H), 7.10(s, 1H), 6.95 (s, 2H), 3.91-3.86 (m, 15H), 3.81–3.76 (m, 4H), 3.58 (t, *J* = 5.76 Hz, 2H), 2.85 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.86, 167.45, 165.38, 164.84, 155.74, 153.60, 150.72, 142.48, 136.20, 130.25, 125.75, 119.6, 99.32, 72.45, 66.27, 61.49, 56.32, 45.92, 43.29, 42.71, 28.76. ESI-MS (m/z): calcd. for $C_{27}H_{32}N_8O_5$ 548.25, found 548.59 $[M + H]^+$.

(4-methylpiperazin-1-yl)(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)methanone (**31e**)

Brown solid; yield: 92%, 0.35g, m.p. 166-168 °C; IR (KBr, cm^{-1}) 3435, 3040, 1680, 1275, 1185, 1125. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (br, 1H), 7.15(s, 1H), 6.95 (s, 2H), 4.20 (t, *J* = 4.5 Hz,

2H), 3.89-3.83 (m, 13H), 3.78 – 3.72 (m, 4H), 3.42 (t, *J* = 5.6 Hz, 4H), 3.23-3.18 (m, 4H), 2.68-2.62 (m, 4H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.90, 164.73, 164.20 (d, *J* = 15.3 Hz), 153.15, 134.96, 133.71, 131.43, 129.50, 98.65, 77.39, 76.75, 66.70, 61.04, 55.98, 47.75, 46.77, 44.22, 43.67, 25.77. ESI-MS (m/z): calcd. for $C_{27}H_{38}N_8O_5$, 554.30, found 554.64 $[M + H]^+$.

(4-ethylpiperazin-1-yl)(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)methanone (**31f**)

Brown solid; yield: 87%, 0.34g, m.p. 132-134 °C; IR (KBr, cm^{-1}) 3430, 3039, 1682, 1274, 1185, 1122. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (br, 1H), 7.10(s, 1H), 6.92 (s, 2H), 4.16 (t, *J* = 4.3 Hz, 2H), 3.86-3.81 (m, 13H), 3.75 – 3.70 (m, 4H), 3.39 (t, *J* = 5.3 Hz, 2H), 2.65-2.59 (m, 4H), 2.38-2.31 (m, 6H), 2.19 (q, *J* = 4.52 Hz, 2H), 1.27 (t, *J* = 3.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 164.52, 163.70 (d, *J* = 15.1 Hz), 153.02, 134.86, 133.51, 131.23, 129.24, 98.25, 77.14, 76.45, 66.35, 60.74, 55.74, 47.25, 46.25, 44.12, 43.25, 25.32, 15.6. ESI-MS (m/z): calcd. for $C_{28}H_{40}N_8O_5$, 568.31, found 568.67 $[M + H]^+$.

N-(4-fluorophenyl)-4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridine-1(2H)-carboxamide (**31g**)

Brown solid; yield: 95%, 0.37g, m.p. 238-239 °C; IR (KBr, cm^{-1}) 3435, 3045, 1680, 1275, 1180, 1120, 1035. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.82 (d, *J* = 8.32 Hz, 2H), 7.43 (d, *J* = 8.15 Hz, 2H), 7.25 (br, 1H), 7.20 (s, 1H), 7.06 (s, 2H), 3.91 (m, 15H), 3.82 – 3.77 (m, 4H), 3.29 (t, *J* = 5.73 Hz, 2H), 2.78 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.84, 166.21, 165.38, 156.91, 154.78, 136.91, 134.55, 133.81, 131.42, 129.78, 128.67, 127.76, 98.61, 77.42, 77.13, 76.81, 66.75, 61.15, 45.39, 43.71, 42.98, 27.50.ESI-MS (m/z): calcd. for $C_{28}H_{32}N_6O_5F$ 565.24, found 565.60 $[M + H]^+$.

N-(3,4-difluorophenyl)-4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridine-1(2H)-carboxamide (**31h**)

Brown solid; yield: 87%, 0.35g, m.p. 135-137 °C; IR (KBr, cm^{-1}) 3445, 3040, 1685, 1270, 1185, 1120, 1040. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.56-7.51 (m, 1H), 7.47-7.43 (m, 1H), 7.32 (d, *J* = 8.34 Hz, 1H), 7.29 (br, 1H), 7.22 (s, 1H), 7.10 (s, 2H), 3.93 (m, 15H), 3.85- 3.81 (m, 4H), 3.31

(t, *J* = 5.75 Hz, 2H), 2.80 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.89, 166.21, 165.42, 156.97, 155.78, 142.35, 141.50, 137.20, 134.65, 133.92, 131.58, 129.95, 128.74, 127.83, 122.6, 116.2, 114.53, 98.76, 76.89, 66.84, 61.24, 45.47, 43.89, 42.76, 27.84. ESI-MS (m/z): calcd. for $C_{28}H_{31}N_7O_5F_2$ 583.24, found 583.6 $[M + H]^+$.

N-(4-methoxyphenyl)-4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridine-1(2H)-carboxamide (**31i**)

Brown solid; yield: 97%, 0.39g, m.p. 166-168 °C; IR (KBr, cm^{-1}) 3430, 3045, 1680, 1275, 1185, 1120. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm, 7.62 (d, *J* = 8.46 Hz, 2H), 7.35 (d, *J* = 8.17 Hz, 2H), 7.24 (br, 1H), 7.17(s, 1H), 6.96 (s, 2H), 3.89-3.86 (m, 15H), 3.78-3.74 (m, 4H), 3.52 (s, 3H), 3.29 (t, *J* = 5.70 Hz, 2H), 2.75 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.42, 167.50, 164.73, 163.24, 155.38, 153.19, 143.79, 134.89, 134.52, 133.79, 133.1, 129.76, 128.65, 127.75, 97.83, 77.41, 77.12, 76.79, 66.73, 61.13, 56.12, 45.37, 43.68, 42.93, 23.75. ESI-MS (m/z): calcd. for $C_{29}H_{35}N_7O_6$ 577.26, found 577.63 $[M + H]^+$.

N-(4-chlorophenyl)-4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridine-1(2H)-carboxamide (**31j**)

Brown solid; yield: 86%, 0.35g, m.p. 174-175 °C; IR (KBr, cm^{-1}) 3430, 3040, 1685, 1270, 1185, 1120, 755. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 4H), 7.21(br, 1H), 7.19 (s, 1H), 6.95 (s, 2H), 4.14 (t, *J* = 10.1 Hz, 2H), 3.87-3.82 (m, (15H), 3.77 (s, 4H), 2.72 (d, *J* = 28.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.52, 167.71, 164.75, 155.4, 153.18, 135.96, 134.79, 134.30, 133.86, 129.16 – 128.45 (m), 128.45 – 128.13 (m), 98.72, 77.38, 77.06, 76.75, 66.70, 61.05, 56.00, 43.70, 31.93, 29.71, 25.76, 25.41. ESI-MS (m/z): calcd. for $C_{28}H_{32}ClN_7O_5$, 581.22, found 582.05 $[M + H]^+$.

(4-(4-morpholino-6-((3,4,5-trimethoxyphenyl)amino)-1,3,5-triazin-2-yl)-5,6-dihydropyridin-1(2H)-yl)(4-(p-tolyl)piperazin-1-yl)methanone (**31k**)

Brown solid; yield: 84%, 0.37g, m.p. 176-178 °C; IR (KBr, cm^{-1}) 3435, 3044, 1685, 1270, 1180, 1125. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.23 Hz, 2H), 7.32 (d, *J* = 8.11 Hz, 2H), 7.22 (br, 1H), 7.18(s, 1H), 6.95 (s, 2H), 4.21 (t, *J* = 4.5 Hz, 2H), 3.92-3.86 (m, 13H), 3.75 – 3.71 (m, 4H), 3.41 (t, *J* = 5.6 Hz, 4H), 3.22-3.17 (m, 4H), 2.64-2.59 (m, 4H), 2.32 (s, 3H). ^{13}C NMR (101

MHz, $CDCl_3$) δ 170.92, 167.35, 164.71, 164.20, 153.15, 137.46, 134.96, 133.71, 131.43, 130.52, 129.50, 127.29, 119.68, 98.65, 77.24, 76.15, 66.72, 61.04, 55.98, 52.73, 47.75, 46.77, 44.22, 43.67, 21.45. ESI-MS (m/z): calcd. for $C_{33}H_{42}N_8O_5$, 630.33, found 630.74 $[M + H]^+$.

6.4.2. Biology

The cell lines, HeLa, Hep G2, A549 and MCF 7 (cervical, liver, lung and breast cancer) which were used in this study were procured from American Type Culture Collection (ATCC), United States. The synthesized test compounds were evaluated for their *in vitro* antiproliferative activity in these four different human cancer cell lines. A protocol of 48h continuous drug exposure was used, and a SRB cell proliferation assay was used to estimate cell viability or growth. All the cell lines were grown in Dulbecco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO2 at 37 °C). Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and seeded in 96-well plates in 100 µL aliquots at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5% CO2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs and were incubated for 48 hrs with different doses (0.01, 0.1, 1, 10, ,100µM) of prepared derivatives**.** After 48 hours incubation at 37 °C, cell monolayers were fixed by the addition of 10% (wt/vol) cold trichloroacetic acid and incubated at 4 °C for 1h and were then stained with 0.057% SRB dissolved in 1% acetic acid for 30 min at room temperature. Unbound SRB was washed with 1% acetic acid. The protein–bound dye was dissolved in 10mM Tris base solution for OD determination at 510 nm using a microplate reader (Enspire, Perkin Elmer, USA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$$[(Ti-Tz)/(C-Tz)] \times 100 \text{ for concentrations for which } Ti \geq Tz$$

$$[(Ti-Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz$$

The dose response parameter, GI_{50} was calculated for each experimental agent. Growth inhibition of 50 % (GI_{50}) was calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, which is the drug

concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Values were calculated for this parameter if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

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Chapter VII

Synthesis of novel phenanthridinyl piperazine triazoles via click chemistry as antiproliferative agents

Chapter 7

Synthesis of novel phenanthridinyl piperazine triazoles via click chemistry as antiproliferative agents

7.1. Introduction

Quinoline skeleton acquired significant interest, owing to its niche in the drug discovery arena. Quinoline derivatives exhibit broad biological spectrum such as anticancer [1], antimalarial, antibacterial [2], anti-HIV [3], antiprotozoal [4], antimycobacterial [5]. Anticancer drugs with quinoline backbone prevailing in the market dofequidar and TAS-103 are depicted in **Figure 7.1**. Quinoline compounds are identified to possess anticancer property by intercalation or alkylation of deoxyribonucleic acid. Targeting this pathway was found to be unsuccessful as the compounds lack selectivity and exhibit broad spectrum of activity. However, it was justified that the selectivity was greatly dependent on the appropriate substituent at the 2nd position of quinoline [6].

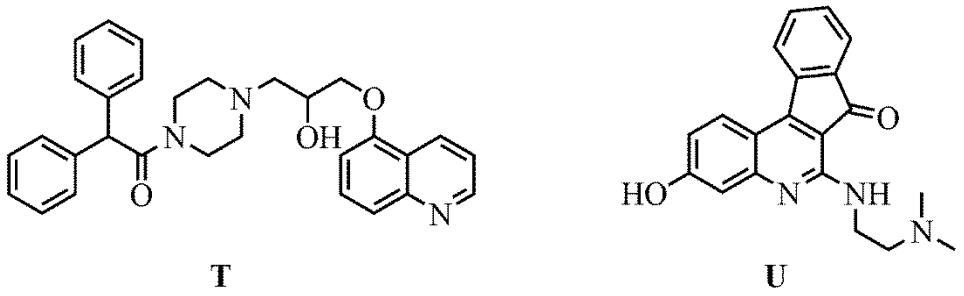


Figure 7.1: Structure of anticancer drugs with quinoline backbone: (T) Dofequidar (U) TAS-103

Also, currently available drugs in the market lack selectivity against normal and tumor cells. Consequently, worsening the treatment of primary or secondary resistance mechanisms evolved in the cancer cells [7].

Some of the quinoline and 1,2,3-triazole containing molecules which exhibit anticancer activity are depicted in **Figure 7.2**.

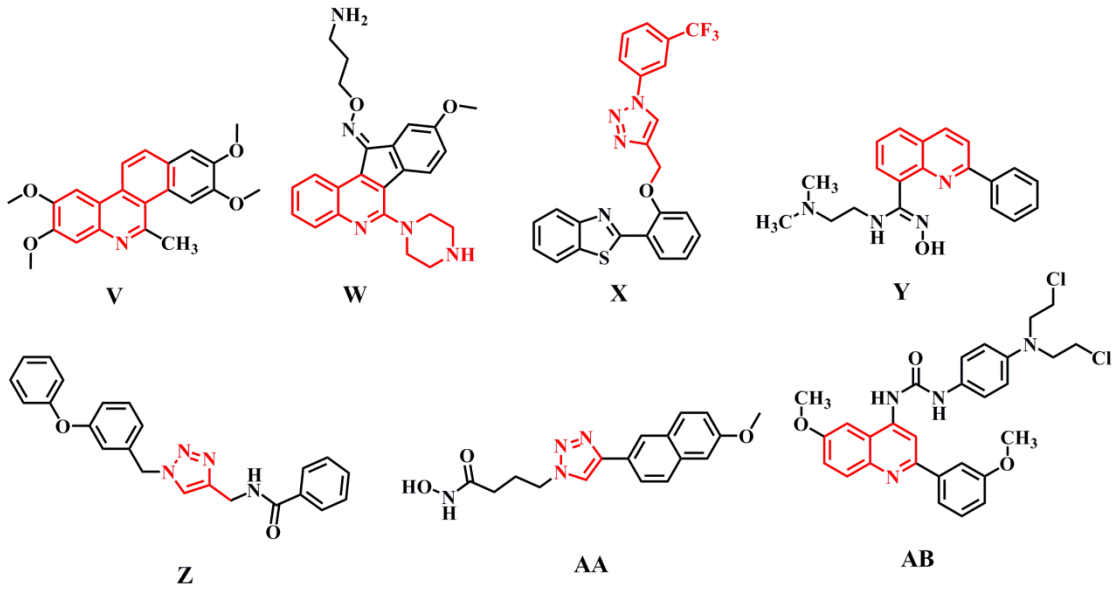
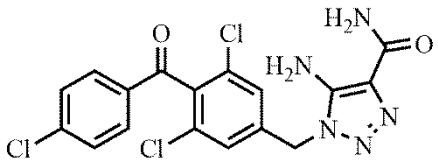


Figure 7.2: Some of the quinoline and 1,2,3-triazole containing molecules which exhibit anticancer activity

On the other hand 1,2,3-Triazoles being imperative and proficient pharmacophore, have occupied chief role not only in organic chemistry but also in medicinal chemistry due to their ease of synthesis by click chemistry with striking chemotherapeutic features covering broad spectrum of biological activities [8, 9, 10]. In particular, carboxyamidotriazole (**AC**) (**Figure 7.3**) is an anticancer drug, containing triazole moiety with potential antineoplastic activity. 1,2,3-triazole ring serves two purposes: (a) it facilitates stronger cap group interactions with the amino acid side chains at the entrance of the histone deacetylase active site; (b) it also serves as bioisostere to the pharmacokinetically and toxicologically disadvantageous groups such as amide and ketone [11,12]. The insertion of 1,2,3-triazole ring which led to the synthesis of *N*-((1-(3-phenoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-phenyloxazole-4-carboxamide is found to be more potent with an IC_{50} of 46 nM against MCF-7 cancer cell line compared to the compounds which lack 1,2,3-triazole moiety [13].



AC

Figure 7.3: Structure of anticancer drug Carboxyamidotriazole

Makhey *et al.*, synthesized 2,3,8,9-tetramethoxy-5-methylbenzo[*i*]phenanthridine (**XX**) which exhibited IC_{50} of 22 and 11µM against the growth of RPMI8402 and CPT-K5 cell lines respectively [14]. Tseng *et al.*, synthesized indeno[1,2-c]quinoline derivatives **(XY)** appended with piperazine at 6th position which turned out to be most potent with GI_{50} values of 0.52, 0.74, 6.76, and 0.64 µM against the growth of HeLa, SKHep, AGS, and A549 cells respectively [15]. Kumbhare *et al.*, synthesized 2-(2-((1-(3-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) benzo[*d*]thiazole **(XZ)** and reported IC_{50} of 11 µM in colon cancer cells [12]. Inspired by the biological importance of 1,2,3-triazoles and quinoline as anticancer agents, we chalked out a trajectory to incorporate these two active pharmacophores. This impelled us to design new chemical entities emphasizing hybrid approach (**Figure 7.4**) anticipating attractive drug scaffold features with important therapeutic potential. Hence, highlighting the importance of substituent at 2nd position of quinoline we coupled 6-(4-(prop-2-ynyl)piperazin-1-yl)phenanthridine with aryl and aryl sulfonyl azides and wanted to explore the synergistic effect of these heterocycles towards anticancer activity. Altogether we report phenanthridinyl piperazine triazoles as novel antiproliferative agents for the first time.

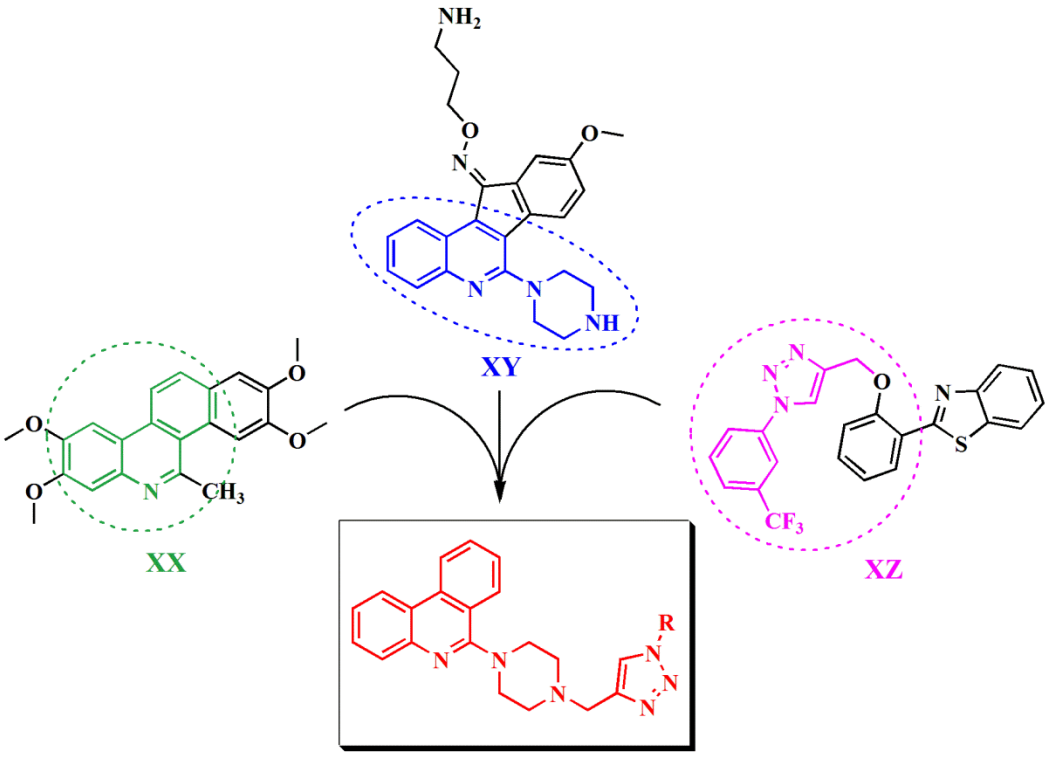
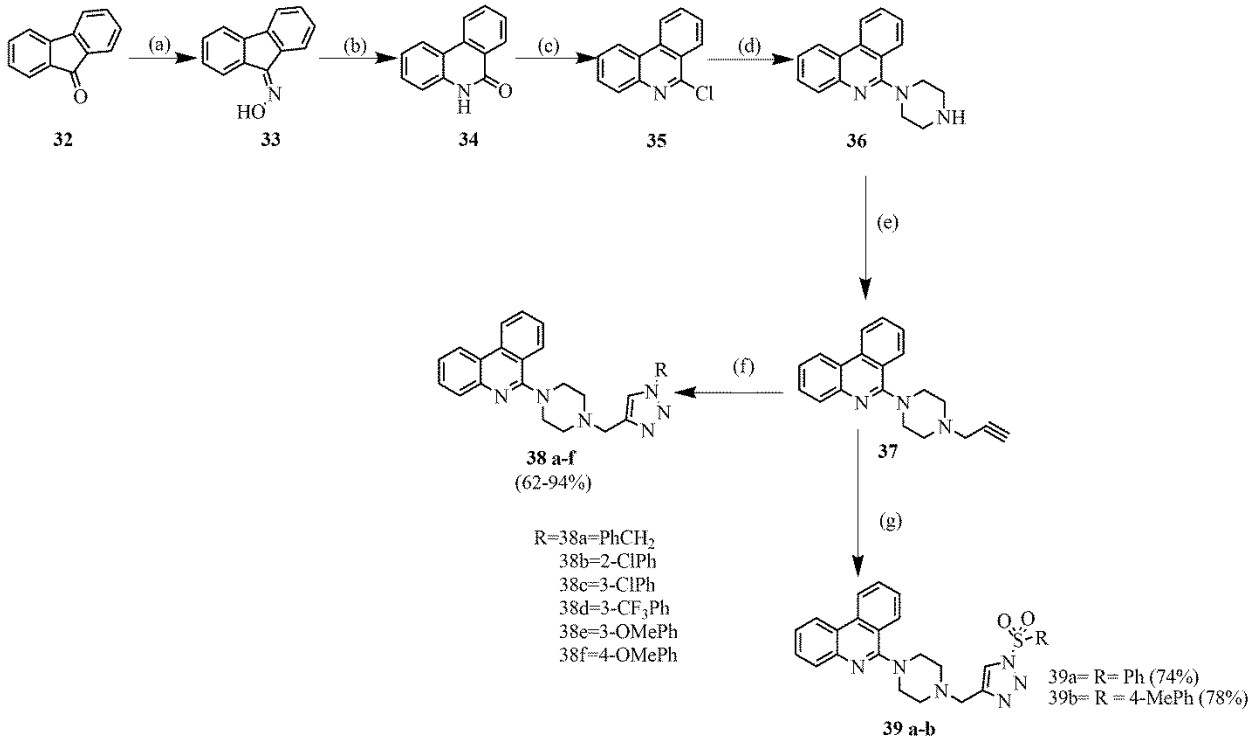


Figure 7.4: Design strategy to achieve title compounds

7.2. Results and discussion

7.2.1. Chemistry

The synthetic route to achieve title compounds is depicted in **scheme 9**.



Scheme 9: Synthetic route to achieve title compounds

Reagents and conditions: (a) $NH_2OH.HCl$ (2equiv), NaOAc (2equiv), EtOH:H_2O (3:1), reflux 1.5h, (b) PPA (10 equiv), P_2O_5 (0.5equiv), heating at 150 °C, 0.5h, (c) $POCl_3$ (10equiv), *N*,*N*-dimethylaniline (0.5equiv), reflux 3h, (d) anhydrous piperazine (3equiv), Et_3N (1.5equiv), DMF, MW, 150 °C, 20 min, (e) propargyl bromide (80% in toluene) (1.2equiv), Et_3N (1.5equiv), DMF, heating at 70 °C 1.5h, (f) substituted azides, $CuSO_4.5H_2O$ (10mol%), sodium ascorbate (10mol%), H_2O:*t*BuOH (1:2), RT 3h, (g) substituted sulfonyl azides, CuTC (10mol%), toluene, RT, 1h.

To generate a novel template which could serve as effective ligand for antiproliferative activity, we adopted reported procedure [16, 17] with slight modification starting from 9-fluorenone (**32**) to prepare 6-Chlorophenanthridine **(35)**, then 6-(piperazin-1-yl) phenanthridine

(36) was synthesized by treating **35** with anhydrous piperazine in DMF under microwave irradiation at 150^{o}C for 20 min using Biotage initiator with a pre-stirring of 30 s and stirring rate at 600 rpm. Compound **37** was obtained by heating **36** with propargyl bromide (80% in toluene) in the presence of triethylamine (TEA) using *N*,*N*-dimethylformamide (DMF) as solvent. The title compounds were synthesized from **37** by means of copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) employing catalytic amount of $CuSO_4.5H_2O$ and sodium ascorbate in 1:2 ratio of water and *tert*-butanol to get desired regioselective 1,4-substituted triazole compounds **38a-f**. While catalytic amount of copper(I)-thiophene-2-carboxylate (CuTC) and toluene as solvent was used to synthesize the regioselective 1,4-substituted triazole compounds **39a-b**.

The ^{1}H NMR spectrum of all title compounds displayed multiplet in the range 2.75-2.95 ppm and 3.45-3.65 ppm corresponding to piperazine (-CH_2-) protons, singlet in the range 3.85-4.00 ppm corresponding to methylene proton, and proton of 1,2,3-triazole ring resonated in the range 7.8-8.2 ppm. Both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and HRMS) of all the synthesized compounds were confirmed and employed further for their evaluation in antiproliferative activity.

7.2.2. Antiproliferative activity

All the synthesized compounds were evaluated for their anti-proliferative activity against four cancer cell lines such as THP1 (Human acute monocytic leukemia), Colo205 (human colon carcinoma), U937 (human leukemic monocytic lymphoma) and HL60 (Human promyelocytic leukemia cells) at concentrations between 1 to 100 µg/mL using Etoposide and *N*,*N*-dimethylsulfoxide (DMSO) as positive and negative control respectively. The anti-proliferative activity results are summarized in **Table 7.1**.

It is evident from the results that considerable structure-activity relationship could be drawn for the tested compounds. Substituents at 2nd or 3rd position of phenyl ring could not able to arrest the cancer cell growth against all the test cell lines **(38b-e)**. Moderate activity was noticed against HL60 cancer cell line when we introduced methylene linker between triazole and aryl ring (**38a**, IC_{50} = 48.98 ± 3.46µg/mL).

Table 7.1: Antiproliferative activity of phenanthridinyl triazole derivatives against different cancerous cell lines THP1, Colo205, U937 & HL60

Compound ID	R	THP1	COLO205	U937	HL60
		IC_{50} (µg/mL)			
38a	$PhCH_2$	--	--	--	48.98 ± 3.46
38b	2-ClPh	--	--	--	--
38c	3-ClPh	--	--	--	--
38d	3-CF_3Ph	--	--	--	--
38e	3-OMePh	--	--	--	--
38f	4-OMePh	--	10.69 ± 1.10	--	92.38 ± 14.68
39a	Ph	4.71 ± 1.98	8.99 ± 1.20	33.26 ± 3.13	8.57 ± 0.63
39b	4-MePh	5.62 ± 0.43	8.73 ± 1.73	19.98 ± 1.56	**3.60 ± 0.16**
Etoposide		2.16 ± 0.1	6.25 ± 0.24	6.04 ± 0.12	8.3 ± 0.32

-- indicates not active at 100 µg/mL

However, compound **38f** exhibited good activity (IC_{50} = 10.69 ± 1.10µg/mL) on Colo 205 and significant activity (IC_{50} = 92.38 ± 14.68µg/mL) on HL60 cancer cell lines when appended with the methoxy group at 4^{th} position of phenyl ring. With this encouraging result in hand, we sandwiched sulfonyl group between triazole and aryl ring to fetch compounds **39a** and **39b**. These derivatives have shown significant decrease in cell viability against all the test cell lines on concentration dependent manner (Table 1). Among the test cell lines, **39a** exhibited excellent activity against HL60, THP1, and Colo205 cancer cell lines with IC_{50} of 8.57 ± 0.63, 4.71 ± 1.98 and 8.99 ± 1.20µg/mL respectively, followed by moderate activity against U937 cancer cell line (IC_{50}= 33.26 ± 3.13 µg/mL). While, **39b** exhibited good activity against Colo205 and THP1 cell lines (IC_{50}= 8.73 ± 1.73 and 5.62 ± 0.43µg/mL respectively), followed by moderate activity against U937 cell line (IC_{50}= 19.98 ± 1.56µg/mL). It is noteworthy that compound **39b** emerged as promising anticancer agent against HL60 cancer line with IC_{50}= 3.60 ± 0.16µg/mL indicating more active than the positive control etoposide. In general electron withdrawing groups were found to be inactive, whereas electron releasing group which acts as hydrogen bond acceptor and/or the groups which have hydrophobic interaction were found to exhibit excellent activity.

7.3. Conclusion

A series of eight 6-(4-((substituted-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl) phenanthridine analogues were synthesized by employing environmentally benign CuAAC and evaluated for their antiproliferative activity in different types of cell lines (THP1, Colo205, U937 & HL60). The differential activity among the cell lines may be accounted to the substituent attached to nitrogen atom of 1,2,3-triazole ring. Influxion of sulfonyl functional group led to the discovery of **39b**, which emerged as more potent than the positive control etoposide with IC_{50}= 3.60 ± 0.16µg/mL against HL60 cancer cell line. These encouraging results promote us to further explore by structural modification on these derivatives which could lead to promising anticancer agents. For the first time we report phenanthridinyl piperazine as new heterocyclic moiety with anticancer property. This study opens up researchers to exploit this heterocycle for lead optimisation and further development of novel anticancer agents.

7.4. Experimental section

7.4.1. Chemistry

Chemicals and solvents were procured from commercial sources and are analytically pure. Thin-layer chromatography (TLC) was carried out on aluminium-supported silica gel plates (Merck 60 F254) with visualization of components by UV light (254 nm). Column chromatography was carried out on silica gel (Merck 230-400 mesh). ^{1}H NMR spectra and ^{13}C NMR spectra were recorded at 400 MHz using a Bruker AV 400 spectrometer (Bruker CO., Switzerland) in $CDCl_3$ solution with tetramethylsilane as the internal standard, and chemical shift values (δ) were given in ppm. Microwave reactions were performed in closed vessel using Biotage Initiator microwave synthesizer (Uppsala, Sweden). Melting points were determined on an electro thermal melting point apparatus (Stuart-SMP30) in open capillary tubes and are uncorrected. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. Elemental analysis was carried out on Elementar (vario MICRO cube, Hanau, Germany).

Synthesis of 6-(piperazin-1-yl)phenanthridine **(36)**: 6-chlorophenanthridine (2.34 mmol) was dissolved in DMF (5 mL) in an oven dry microwave vial. Then TEA (3.51 mmol) followed by anhydrous piperazine (4.68 mmol) were added. Microwave vial was sealed with aluminium cap and the resultant mixture was subjected to microwave irradiation at 150 °C for 20 min.

Completion of the reaction was monitored by TLC using 10% MeOH in DCM as mobile phase. After the reaction was complete, DMF was evaporated under vacuo and added 5 mL of water. Compound was extracted using EtOAc (3 x 5mL). Combined organic layers were washed with saturated brine solution, dried over anhydrous sodium sulphate and evaporated in vacuo. Column chromatography of the residue using gradient 5% MeOH in DCM gave 6-(piperazin-1-yl)phenanthridine. yellow solid, Yield 62%, 0.32g, mp 116-119 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.57 (1H, d, *J* = 8.2 Hz, 1H), 8.45 (d, *J* = 7.9 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 2H), 7.49 (t, *J* = 7.6 Hz, 1H), 4.81 (s, br 1H). 3.82 – 3.25 (m, 8H).^{13}C NMR (DMSO-d_6, 101MHz) δ 171.87, 147.68, 136.79, 134.12, 129.97, 128.32, 126.11, 125.56, 124.13, 122.86, 121.64, 120.76, 113.78, 51.23, 46.69. ESI-MS (m/z): calcd. for $C_{17}H_{18}N_3$ 264.15, found 265.23 $(M+H)^+$.

Synthesis of 6-(4-(prop-2-ynyl)piperazin-1-yl)phenanthridine **(37)**: 6-(piperazin-1-yl)phenanthridine (0.0187 mol) was dissolved in DMF (50 mL), then TEA (0.0280 mol) followed by propargyl bromide (80% in toluene) (0.0280 mmol) were added. Resultant mixture was heated at 70 °C for 1.5h. Completion of the reaction was monitored by TLC using 5% MeOH in DCM as mobile phase. After the reaction was complete, DMF was evaporated in vacuo and added 50 mL of water. Compound was extracted using EtOAc (3x15mL). Combined organic layers were washed with saturated brine solution, dried over anhydrous sodium sulphate and evaporated in vacuo. Column chromatography of the residue using 1-2% MeOH in DCM gave 6-(4-(prop-2-ynyl)piperazin-1-yl)phenanthridine. Pale yellow solid, yield=92%, 0.23g, m.p. 125-126 ^{o}C; ^{1}H NMR (400MHz, $CDCl_3$) δ 8.46 (d, *J* = 8.0 Hz, 1H), 8.36 (d, *J* = 7.6, Hz, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 9.6 Hz, 1H), 7.76 – 7.39 (m, 4H),3.94 (s, 2H), 3.58 (t, *J* = 6.6, 4H), 2.92 (t, *J* = 6.8, 4H), 2.42 (s, 1H), . ^{13}C NMR (101MHz, $CDCl_3$) δ 174.27, 138.62, 134.53, 131.65, 129.44, 128.33, 127.45, 125.34, 124.56, 123.65,122.64, 121.16, 117.76, 78.64, 76.89, 58.72, 56.21 50.63. ESI-MS (m/z): calcd. for $C_{20}H_{20}N_3$ 302.16, found 303.23 $(M+H)^+$.

*Synthesis of 6-(4-((substituted-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine***(38a-f)** 6-(4-(prop-2-ynyl)piperazin-1-yl)phenanthridine (0.6571 mmol) was dissolved in 1:2 ratio of water and *t*BuOH (3 mL). Then $CuSO_4.5H_2O$ (0.1314 mmol), sodium ascorbate (0.1314 mmol) and aryl azides (0.7228 mmol) was added. Resultant mixture was stirred at RT for 3h.

Completion of the reaction was monitored by TLC using 2% MeOH in DCM as mobile phase. After the reaction was complete, volatile was evaporated in vacuo and the compound was extracted using EtOAc (3 x 5mL). Combined organic layers were washed with saturated brine solution, dried over anhydrous sodium sulphate and evaporated in vacuo. Column chromatography of the residue using 1-2% MeOH in DCM gave regioselective 1,4-substituted title compounds.

6-(4-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine **(38a)** White solid; yield: 91%, 0.24g, m.p. 132-133 °C; IR (KBr, cm^{-1}) 3025, 1650, 1210. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.2, Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.88 (d, *J* = 9.2 Hz, 1H), 7.78 – 7.32 (m, 9H), 4.98 (s, 2H), 3.92 (s, 2H), 3.55 (t, *J* = 7.3 Hz, 4H), 2.90 (t, *J* = 6.9 Hz, 4H). ^{13}C NMR (101MHz, $CDCl_3$) δ 172.46, 148.76, 141.28, 139.84, 138.32, 135.51, 134.42, 130.80, 129.47, 128.13, 127.69, 126.61, 124.60, 123.11, 122.24, 121.64, 120.43, 119.92, 116.85, 60.16, 58.18, 50.74, 45.27. ESI-MS (m/z): calcd. for $C_{27}H_{27}N_6$ 435.22, found 436.28 $(M+H)^+$.

6-(4-((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (**38b**)
Pale yellow solid; yield: 82%, 0.27g, m.p. 122-123 °C; IR (KBr, cm^{-1}) 3014, 1656, 1145, 735. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.35 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.96 (d, *J* = 9.2 Hz, 1H), 7.74 (d, *J* = 7.2 Hz, 1H), 7.68 (d, *J* = 9.2 Hz, 1H), 7.58 – 7.36 (m, 6H), 3.92 (s, 2H), 3.55 (t, *J* = 7.2 Hz, 4H), 2.90 (t, *J* = 6.3 Hz, 4H). ^{13}C NMR (101MHz, $CDCl_3$) δ 173.42, 149.76, 142.36, 140.58, 139.84, 137.84, 135.51, 130.80, 129.47, 128.42, 128.13, 127.92, 127.69, 126.61, 125.60, 124.72, 123.11, 121.64, 120.43, 119.92, 115.85, 58.18, 50.74, 45.27. ESI-MS (m/z): calcd. for $C_{26}H_{24}ClN_6$ 455.17, found 456.23 $(M+H)^+$.

6-(4-((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (**38c**)
White solid; yield: 94%, 0.31g, m.p. 121-122 °C; IR (KBr, cm^{-1}) 3013, 1643, 1156, 748. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.48 (d, *J* = 8.4 Hz, 1H), 8.44 (d, *J* = 7.6, Hz, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 8.12 –8.00 (s, 2H),7.93 (d, *J* = 9.2 Hz, 1H), 7.84 (d, *J* = 7.2 Hz, 1H), 8.81 (d, *J* = 7.6 Hz, 1H), 7.78 – 7.46 (m, 5H), 3.92 (s, 2H), 3.55 (t, *J* = 6.9 Hz, 4H), 2.90 (t, *J* = 6.8 Hz, 4H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 173.12, 151.76, 144.35, 142.58, 139.84, 137.84, 135.51, 130.80,

129.47, 128.13, 127.69, 126.61, 125.60, 124.12, 123.11, 122.88 121.64, 120.43, 119.92, 118.85, 116.36, 58.18, 50.74, 45.27. ESI-MS (m/z): calcd. for $C_{26}H_{24}ClN_6$ 455.17, found 456.22 $(M+H)^+$.

6-(4-((1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (**38d**)

Yellow solid; yield: 85%, 0.22g, m.p. 130-131 °C; IR (KBr, cm^{-1}) 3045, 1635, 1240, 1120. ^1H NMR (400MHz, $CDCl_3$) δ 8.57 (d, *J* = 8.4 Hz, 1H), 8.36 (d, *J* = 7.2, Hz, 1H), 8.21 (d, *J* = 7.6 Hz, 1H), 8.10 –8.00 (s, 2H),7.98 (d, *J* = 9.6 Hz, 1H), 7.94 (d, *J* = 7.2 Hz, 1H), 8.86 (d, *J* = 7.6 Hz, 1H), 7.76 – 7.39 (m, 5H), 3.92 (s, 2H), 3.55 (t, *J* = 6.8 Hz, 4H), 2.90 (t, *J* = 7.0 Hz, 4H). ^{13}C NMR (101MHz, $CDCl_3$) δ 173.93, 150.16, 143.45, 142.58, 138.44, 137.84, 135.51, 130.80, 129.47, 128.13, 127.69, 126.61, 125.60,124.89, 124.12,123.11, 122.88 121.64, 121.13, 119.62, 118.42, 116.76, 58.78, 50.64, 45.36. ESI-MS (m/z): calcd. for $C_{27}H_{24}F_3N_6$ 489.20, found 490.28 $(M+H)^+$.

6-(4-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (**38e**)

Pale yellow semi solid; yield: 68%, 0.21g, m.p. oily mass; IR (KBr, cm^{-1}) 3041, 1630, 1215, 1090. ^1H NMR (400MHz, $CDCl_3$) δ 8.46 (d, *J* = 8.0 Hz, 1H), 8.36 (d, *J* = 7.6, Hz, 1H), 8.21 (d, *J* = 7.2 Hz, 1H), 8.00 (s, 1H),7.92 (d, *J* = 9.6 Hz, 1H), 7.90 (d, *J* = 7.2 Hz, 1H), 7.76 – 7.39 (m, 5H), 6.96 (d, *J* = 7.6 Hz, 1H), 6.92 (s, 1H), 3.99 (s, 3H), 3.94 (s, 2H), 3.58 (t, *J* = 6.8 Hz, 4H), 2.92 (t, *J* = 6.9 Hz, 4H). ^{13}C NMR (101MHz, $CDCl_3$) δ 174.27, 150.45, 142.23, 141.28, 138.62, 136.45, 134.53, 131.65, 129.44, 128.33, 127.45, 126.89, 125.34, 124.56, 123.65, 122.64 121.32, 121.16, 119.42, 118.12, 117.76, 58.72, 56.21 50.63, 45.38. ESI-MS (m/z): calcd. for $C_{27}H_{27}N_6O$ 451.22, found 452.25 $(M+H)^+$.

6-(4-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine (**38f**)

Pale green solid; yield: 78%, 0.32g, m.p. 128-129 °C; IR (KBr, cm^{-1}) 3072, 1624, 1228, 1123. ^1H NMR (400MHz, $CDCl_3$) δ 8.56 (d, *J* = 8.4 Hz, 1H), 8.42 (d, *J* = 7.2, Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.68 – 7.37 (m, 4H),6.92 (d, *J* = 7.2 Hz, 2H), 3.98 (s, 3H). 3.92 (s, 2H), 3.58 (t, *J* = 6.9 Hz, 4H), 2.93 (t, *J* = 6.7 Hz, 4H). ^{13}C NMR (101MHz, $CDCl_3$) δ 174.10, 162.42, 149.72, 140.58, 139.84, 138.84, 135.51, 134.80,

129.47, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 121.12, 119.62, 118.45, 115.46, 62.34, 58.98, 51.74, 46.27. ESI-MS (m/z): calcd. for $C_{27}H_{27}N_6O$ 451.22, found 452.28 $(M+H)^+$.

Synthesis of 6-(4-((substituted-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine **(39a-b)**

6-(4-(prop-2-ynyl)piperazin-1-yl)phenanthridine (0.6571 mmol) was dissolved in toluene (5 mL). Then CuTC (0.0657 mmol), and sulfonyl azides (0.7228 mmol) was added. Resultant mixture was stirred at RT for 2h. Completion of the reaction was monitored by TLC using 2% MeOH in DCM as mobile phase. After the reaction was complete, saturated aq NH_4Cl (5 mL) was added and the compound was extracted using EtOAc (3 x 5mL). Combined organic layers were washed with saturated brine solution, dried over anhydrous sodium sulphate and evaporated in vacuo. Column chromatography of the residue using 1-2% MeOH in DCM gave regioselective 1,4-substituted title compounds.

6-(4-((1-benzenesulfonyl-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine **(39a)**

White solid; yield: 74%, 0.28g, m.p. 151-152 °C; IR (KBr, cm^{-1}) 3027, 1655, 1225, 1050. ^1H NMR (400MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H), 7.93 (d, *J* = 9.2 Hz, 1H), 7.78 – 7.32 (m, 9H), 3.92 (s, 2H), 3.54 (t, *J* = 6.6 Hz, 4H), 2.90 (t, *J* = 6.7 Hz, 4H). ^{13}C NMR (101MHz, $CDCl_3$) δ 174.12, 151.76, 142.18, 139.84, 137.84, 135.51, 134.42, 130.80, 129.47, 128.13, 127.69, 126.61, 124.60, 123.11, 121.64, 120.43, 119.92, 114.85, 108.16, 58.18, 50.74, 45.27. ESI-MS (m/z): calcd. for $C_{26}H_{25}N_6O_2S$ 485.17, found 486.24 $(M+H)^+$.

6-(4-((1-tosyl-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)phenanthridine **(39b)**

White solid; yield: 78%, 0.27g, m.p. 101-102 °C; IR (KBr, cm^{-1}) 3025, 1650, 1210, 1045. ^1H NMR (400MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.4 Hz, 1H), 8.43 (d, *J* = 7.6, Hz, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 8.00 (s, 1H),7.93 (d, *J* = 9.2 Hz, 1H), 7.83 (d, *J* = 9.2 Hz, 2H), 7.72 (d, *J* = 7.6 Hz, 2H), 7.68 – 7.46 (m, 4H), 3.92 (s, 2H), 3.55 (t, *J* = 6.6 Hz, 4H), 2.90 (t, *J* = 6.8 Hz, 4H), 2.42 (s, 3H). ^{13}C NMR (101MHz, $CDCl_3$) δ 172.12, 150.76, 142.35, 140.58, 139.84, 138.84, 135.51, 134.80, 129.47, 128.13, 127.69, 126.61, 125.60, 123.11, 121.64, 120.43, 119.92, 118.85, 114.36, 58.18, 50.74, 45.27, 24.64. ESI-MS (m/z): calcd. for $C_{27}H_{27}N_6O_2S$ 499.19, found 500.22 $(M+H)^+$.

7.4.2. Cell lines and cell culture

The cell lines THP1 (Human acute monocytic leukemia), Colo205 (human colon carcinoma), U937 (human leukemic monocytic lymphoma) and HL60 (Human promyelocytic leukemia cells) were obtained from the National Centre for Cellular Sciences (NCCS), Pune, India. Cells were cultured in RPMI-1640 media, supplemented with 10% heat-inactivated foetal bovine serum (FBS), 100 units/ml penicillin and 100µg/ml streptomycin. All cell lines were maintained in culture at 37 °C in an atmosphere of 5% CO_2.

7.4.3. Cytotoxicity

Cell proliferation or viability was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay [18]. Cells were seeded in each well containing 100 µl medium at a final density of 2x104 cells/well, in 96 well micro titer plates at identical conditions. Substituted triazole compounds were dissolved and eventually further diluted in dimethylsulfoxide (DMSO). After overnight incubation, the cells were treated with different test concentrations (1-100µg/mL) or carrier solvent alone in a final volume of 200 µl with five replicates each. The concentration of DMSO did not exceed 0.1%, which is considered non-toxic to cells. After 24 h, 10 µl of MTT (5 mg/mL) was added to each well and the plate was incubated at 37 °C in the dark for 4 h. Supernatants were removed and the formazan crystals were solubilised in DMSO (100 µl/well) for 30 minutes at room temperature. The reduction of MTT was quantified by absorbance at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SOFTmax PRO-5.0). Effects of the test compounds on cell viability were calculated using cells treated with DMSO as control. The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC_{50} (inhibition of cell viability) concentrations were calculated using the respective regression equations.

7.5. References

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Chapter VIII

Summary and Conclusion

Summary and Conclusion Chapter 8

Form literature search we found that there are many good chemical moieties which were inhibiting cancer cells, but they were not turning into potent drug candidates due to many other side effects. We had chosen reported anticancer compounds with good IC_{50} values as lead molecules and redesigned the compounds to get more drug like properties by retaining the core structure for the activity. These leads were taken up for hit expansion by chemical synthesis and analogues from five different series were synthesized, characterized by ^{1}H, ^{13}C, LCMS and evaluated for their antiproliferative activity on various human cancer cell lines.

In **scheme 1**, twenty two new ciprofloxacin analogues were synthesized and evaluated for their antiproliferative activity against acute lymphoblastic leukemiacells (CCRF-CEM), breast adenocarcinoma cells (MDA-MB-468) and human colon carcinoma cells (HCT-116) by cell proliferation assay. Among the synthesized compounds **3t** at 50 μM showed comparable potency to doxorubicin (10 μmol) in all three cell lines, while **3j** inhibited proliferation of MDA-MB-468 up to 35% selectively over other two cell lines. These results reveal the significance of fluoro substituent and further modification to develop anti-cancer agent.

In **scheme 2,** eighteen new ciprofloxacin analogues were synthesized and evaluated for their antiproliferative activity against lung cancer (A549), pancreatic cancer (MiaPaca), cervical cancer (HeLa), breast cancer (MDA MB-231, MCF-7) by MTT assay. Among the synthesized compounds **6o** compound showed better activity to (IC_{50} = 14.21±0.66 μM) doxorubicin. Many of the synthesized compounds do not exhibit toxic effect on normal human embryonic kidney cell line (HEK) compared with doxorubicin. DNA-binding properties of the synthesized **6o** compound investigated by UV-visible and fluorescence spectroscopies clearly denote that the compound can bind to DNA through intercalation mode.

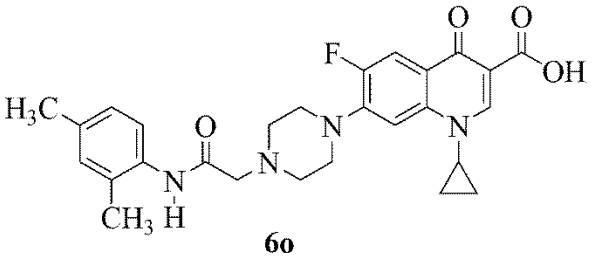
In **scheme 3**, twenty four new **CP**-1,2,3 triazole hybrid analogues were synthesized and evaluated for their antiproliferative activity against ovarian carcinoma cell line (SK-OV-3) and human T cell lymphoblast cell lines (CCRF-CEM). Among all the synthesized compounds, **8b**, **8g**, **8k**, **8r**, **8t** were found to be more active than Dox against CCRF-CEM. Compound **8k** was

found to be more active than Dox against SK-OV-3. DNA binding properties of the synthesized compound **8t** was investigated by UV and fluorescence spectroscopic techniques. Experimental results clearly indicate that the compound **8t** binds to DNA through intercalation mode and might stop DNA replication hence might exhibit better antiproliferative activity than Doxorubicin.

In **scheme 4** and **scheme 5**, twenty eight new 2-(4-aminophenyl)benzothiazole analogues have been synthesized, and evaluated for their growth inhibitory activity against three different human cancer cell lines such as lung cancer (A549), cervical cancer (HeLa) and breast cancer (MDA-MB-231). Among the synthesized compounds, **13f** (GI_{50} = 0.25±0.02 µM), **13g** (GI_{50} = 0.18±0.03 µM), **13j** (GI_{50} = 0.14±0.02 µM), and **15k** (GI_{50} = 0.14±0.02 µM), showed maximum growth inhibitory activity against cancer cell lines at low concentrations. Our findings from this work with synthesis, antiproliferative activity and molecular modeling experiments demonstrate that these 2-(4-aminophenyl)benzothiazole analogues could be potential candidates for developing novel anticancer agents.

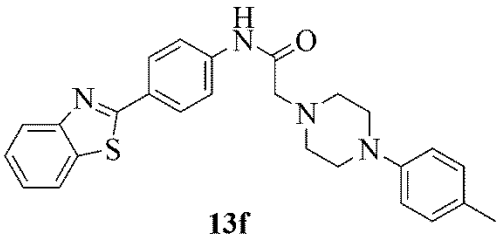
In **scheme 6** and **scheme 7**, thirty two new pyrrolo[2,3-b]pyridine analogues have been and synthesized, and evaluated for their growth inhibitory activity against three different human cancer cell lines such as lung cancer (A549), cervical cancer (HeLa) and breast cancer (MDA-MB-231). Among the synthesized compounds, **20c (**GI_{50} = 0.25±0.03 µM), **20d** (GI_{50} = 0.12±0.01 µM), **20e** (GI_{50} = 0.13±0.01 µM), **20k (**GI_{50} = 0.16±0.04 µM), **20m (**GI_{50} = 0.13±0.01 µM), **20s** (GI_{50} = 0.14±0.02 µM), **22j (**GI_{50} = 0.18±0.02 µM) and **22k (**GI_{50} = 0.17±0.01 µM) showed maximum growth inhibitory activity against cancer cell lines at low concentrations. The specific interaction of compound **20d** (GI_{50} = 0.12±0.01 µM) with calf thymus DNA by intercalate mode, which might further block DNA replication to exert their antiproliferative activity was studied.

In **scheme 8**, thirty seven new 1,3,5-triazine analogues have been and synthesized, and evaluated for their inhibitory activity against four different human cancer cell lines such as HeLa (Cervical cancer), HepG2 (liver carcinoma cancer), A549 (Lung cancer), and MCF 7 (Breast cancer) employing sulforhodamine B (SRB) assay method. Among the synthesized compounds **30j (**IC_{50}= 9.6±0.4 µM) compound exhibited comparable inhibitory activity.



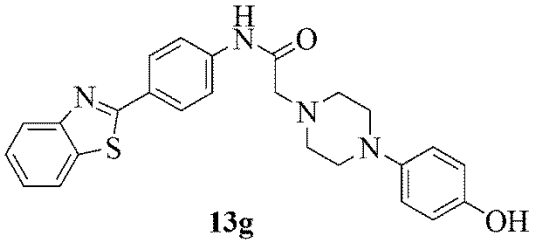
6o

IC_{50} = 14.21 ± 0.66 μM



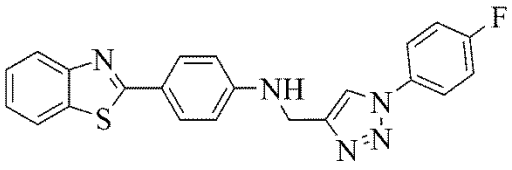
13f

GI_{50} = 0.25±0.02 μM



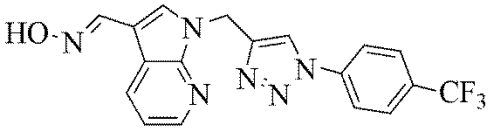
13g

GI_{50} = 0.18±0.03 μM



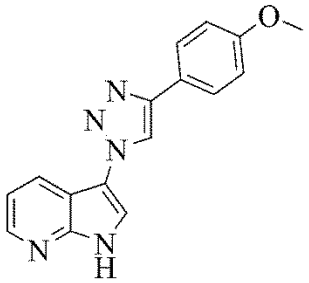
15k

GI_{50} = 0.14±0.02 μM



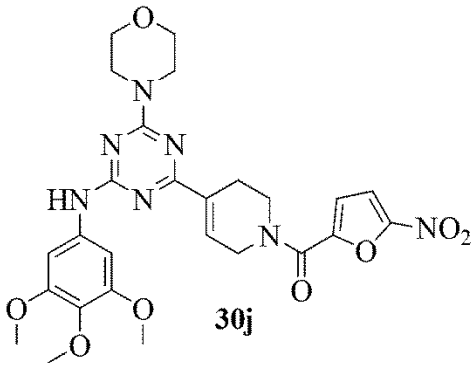
20d

GI_{50} = 0.12±0.01 μM



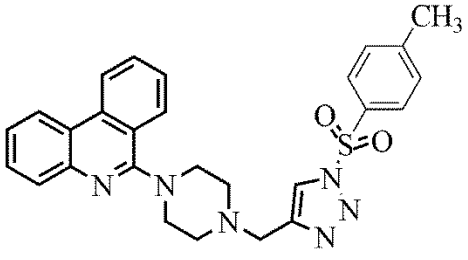
22k

GI_{50} = 0.17±0.01 μM



30j

IC_{50} = 9.6±0.4 μM



39b

IC_{50} = 3.60 ± 0.16 μM

Figure 8.1: Active structures of synthesized compounds

In **scheme 9**, eight new phenanthridine analogues have been synthesized and evaluated for their anti-proliferative activity in different types of cell lines Human acute monocytic leukemia (THP1), human colon carcinoma (Colo205), human leukemic monocytic lymphoma (U937) and Human promyelocytic leukemia cells (HL60). Among the synthesized compounds **39b** more potent than the positive control etoposide with IC_{50}= 3.60 ± 0.16µg/mL against HL60 cancer cell line. These encouraging results promote us to further explore structural modification on these derivatives which could lead to promising anticancer agents. This study opens up researchers to exploit this heterocyclic compound for lead optimization and further development of novel anticancer agents.

In conclusion, the class of compounds depicted here provide promising lead compounds for further drug optimization and development to yield best novel entities aimed to treat cancer. The study also provides the basis for further chemical optimization of these potent inhibitors as anticancer agents.

Future perspectives

- The present thesis describes the development of five various heterocyclic scaffold series of analogs as potential anticancer agents. The synthesized molecules exhibit significant *in vitro* anticancer activity against various cancer cell lines.

- As the anticancer results are encouraging, for lead optimization *in vivo* studies need to be performed to confirm the pharmacodynamic and pharmacokinetic profile of the potent analogues.

- Based on the pharmacophore model proposed, various substituents which lead to anticancer activity proposed could be incorporated into the compounds synthesized and further studied in various animal models.

- For potent anticancer compounds the DNA binding sites can be discovered and the effect on proliferation of cancer cells can be found.

- The present study can be extended to find out at what stage the cell cycle arrest of the active synthesized analogues is happening.

- Extensive side effect profile of active synthesized compounds need to be carried out.

Appendix

List of Publications

From thesis work:

1) **Suresh**, **N**., Nagesh, H.N., Anil Kumar, Shirazi, A.N., Parang, K., Chandra Sekhar, K.V.G. Synthesis of novel ciprofloxacin analogues and evaluation of their anti-proliferative effect on human cancer cell lines. **Bioorg. Med. Chem. Lett. 2013, 23 (23), 6292-6295.**

2) Nagesh, H.N., **Suresh**, **N**., Bhanu Prakash, G.V.S., Gupta, S., Rao, J.V., Chandra Sekhar, K.V.G. Design, synthesis and biological evaluation of novel phenanthridinyl piperazine triazoles via click chemistry as anticancer agents. **Med. Chem. Res. 2015, 24, 523-532.**

3) **Suresh**, **N**., Surendar, C., Sowjanya, p., Mallika, A., Nishant, J., Chandra Sekhar, K.V.G. Synthesis and biological evaluation of pyrrolo[2,3-*b*]pyridine analogues as antiproliferative agents and their interaction with calf thymus DNA (manuscript under review).

4) **Suresh**, **N**., Anil, K., Amir, N.S., Keykavous, P., Mallika, A., Chandra Sekhar, K.V.G. Design, synthesis and biological evaluation of ciprofloxacin-1,2,3-triazole hybrid analogues via click chemistry as antiproliferative agents (manuscript under review).

5) **Suresh**, **N**., Lakshminarayan Reddy, T., Yerramsetty, S., Pal Bhadra, M., Mallika A., Chandra Sekhar, K.V.G. Antiproliferative activity, molecular modeling studies and interaction with Calf thymus DNA of novel ciprofloxacin analogues (manuscript under communication).

6) **Suresh**, **N**., Suresh, A., Surendar, C., Sowjanya, p., Mallika, A., Nishant, J., Chandra Sekhar, K.V.G. Design, synthesis and biological evaluation of 2-(4-aminophenyl)benzothiazole analogues as antiproliferative agents (manuscript under communication).

7) **Suresh**, **N**., Surendar, C., Sowjanya, p., Mallika, A., Nishant, J., Chandra Sekhar, K.V.G. Synthesis and biological evaluation of 1,3,5-triazine analogues as antiproliferative agents (manuscript under communication).

Other publications:

1) **Suresh**, **N**., Nagesh, H.N., Renuka, J., Rajput, V., Sharma, R., Khan, I.A., Chandra Sekhar, K.V.G. Synthesis and evaluation of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-(4-substitutedpiperazin-1-yl)acetyl) piperazin-1-yl)quinoline-3-carboxylic acid derivatives as anti-tubercular and antibacterial agents. **Eur. J. Med. Chem. 2014, 71, 324-332.**

2) Nagesh, H.N., **Suresh**, **N**., Mahalakshmi Naidu, K., Arun, B., Sridevi, J.P., Sriram, D., Yogeeswari, P., Chandra Sekhar, K.V.G. Synthesis and evaluation of anti-tubercular activity of 6-(4-substitutedpiperazin-1-yl) phenanthridine analogues. **Eur. J. Med. Chem. 2014, 74, 333-339**.

3) Chandra Sekhar, K.V.G., Rao. V.S., Tara Sasank, T. V. N. V., Nagesh, H.N., **Suresh**, **N**, Mahalakshmi Naidu, K., Suresh, A., Synthesis of 3,5-diarylisoxazoles under solvent-free conditions using iodobenzene diacetate. **Chin. Chem. Lett. 2013, 24 (12), 1045 -1048**.

Papers presented at Conferences

1) **N. Suresh**, H.N. Nagesh, M.P. Bhadra, M. Alvala, K.V.G. Chandra Sekhar, Synthesis, *in vitro* antiproliferative activity of 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-(substituted phenylamino)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylicacid analogues**.** 16th Tetrahedron symposium, Berlin, Germany, June 16-19th, 2015.

2) **N. Suresh**, A. Kumar, K. Parang, M. Alvala, K.V.G. ChandraSekhar. Synthesis and anticancer evaluation of novel ciprofloxacin analogues. International Conference on Innovations in Chemical Research and Applied Chemical Sciences at Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar-3, Mumbai, January 12-13th, 2015. **(Won the best paper award in poster section).**

3) **N. Suresh**, H.N.Nagesh, K.Mahalaxmi Naidu, A. Suresh, K.V.G. Chandra Sekhar, K. Parang, Design and synthesis of 1-cyclopropyl-6-fluoro-4-oxo-7-{4-substituted piperazin-1-yl}-1, 4-dihydro quinoline-3-carboxylic acid derivatives asAntiproliferative agents. National Poster- II Symposium on Advances in Organic / Medicinal Chemistry (AOMC-2013), Krishna University, Vijayawada, December 21st, 2013.

4) **N. Suresh**, H.N.Nagesh, K.Mahalaxmi Naidu, Inshad Ali Khan, K.V.G. Chandra Sekhar, Design and synthesis of 1-cyclopropyl-6-fluoro-4-oxo-7-{4-substituted piperazin-1-yl}-1, 4-dihydro quinoline-3-carboxylic acid derivatives as Anti-tubercular agents, 2nd UK-India MedChem Congress, IICT Hyderabad, March 22-23rd, 2013.

5) **N. Suresh**, H.N.Nagesh, K.Mahalaxmi Naidu, K.V.G. Chandra Sekhar, Design and synthesis of 1, 3-disubstituted 5-(2-(4-substituted piperazine-1-yl) acetyl) indoline-2-one derivatives as anti-cancer agents, 15th CRSI National Symposium in Chemistry, Banaras Hindu University – Varanasi, February 1-3rd, 2013.

6) **N. Suresh**, H.N.Nagesh, K.Mahalakshmi Naidu, K.V.G. Chandra Sekhar, Design and Synthesis of 1-cyclopropyl-6-fluoro-4-oxo-7-{4-substituted piperazin-1-yl}-1,4-dihydro quinoline-3-carboxylic acid derivatives as anti-tumor agents, 6th Midyear CRSI Symposium in Chemistry, CDRI Lucknow, July 21-22nd, 2012.

Biography of Prof. K.V.G. Chandra Sekhar

Prof. K.V.G. Chandra Sekhar completed his B.Pharm (Hons.) in 1999 from BITS Pilani and after worked as a faculty in Gurukul vidyapeeth junior college, Hyderabad for two years. He re-joined BITS Pilani in 2001 as teaching assistant and completed his M.Pharm in 2003. He then worked as assistant lecturer for one year and then as lecturer up to 2008. He was awarded Ph.D in synthetic medicinal chemistry in 2008. From 2008 to 2014 he worked as assistant professor and currently he is working as associate professor since 2015. His areas of research interest are synthetic medicinal chemistry and drug design. As investigator, he successfully completed major research projects funded by UGC, DST and DBT. He has published over 25 research articles in well renowned international journals and presented around 35 papers in various conferences/symposia and workshops. He is a life member of association of pharmacy teachers of India, CRSI, Indian pharmacological society, Indian council of chemist, Indian association of chemistry teachers etc.

Biography of Mr. Suresh N

Mr. Suresh N completed his BSc (Botany, Zoology and Chemistry) in 2003 from Osmania University. He completed his Bachelor of Education (B.Ed) in 2005 with distinction from Osmania University. He completed his MSc (Organic chemistry) in 2009 from Osmania University. He qualified Joint CSIR-UGC test JRF in 2010-June with 357 rank as a UGC-JRF. He worked as a lecturer in Gauthami P.G College for one year. He joined as a junior research fellow (JRF) in BITS Pilani, Hyderabad campus in 2011-August under the supervision of Prof. K.V.G. Chandra Sekhar. He was promoted as senior research fellow (SRF) in 2013-August. He was awarded ICMR travel grant for international travel support for attending the 16^{th} Tetrahedron symposium in Berlin, Germany. He has published five scientific papers in international journals and presented six papers at national and international conferences and he got best poster award in International Conference on Innovations in Chemical Research and Applied Chemical Sciences at, Mumbai in 2015.