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

SYNOPSIS OF THE THESIS

Submitted in partial fulfilment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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Introduction Chapter 1

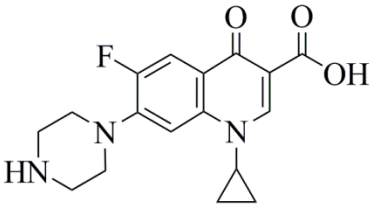
1. General introduction

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.

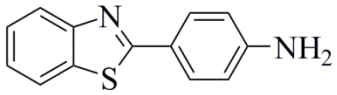
In India, the total cancer cases are expected to go up from 979,786 cases in the year 2010 to 1,148,757 cases in the year 2020. The tobacco-related cancers for males are expected to go up from 190,244 in the year 2010 to 225,241 in the year 2020. Similarly, the female cases will go up from 75,289 in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

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**) We synthesized the analogues and evaluated their anticancer activity by using MTT assay, cell proliferation assay and SRB assay.



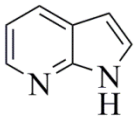
Fluoroquinolone (Ciprofloxacin)

A



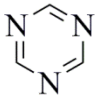
2-(4-aminophenyl)benzothiazole

B



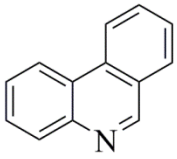
1*H*-pyrrolo[2,3-*b*]pyridine

C



1,3,5-triazine

D



Phenanthridine

E

Figure: Structures of selected heterocyclic scaffolds

Objectives Chapter 2

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

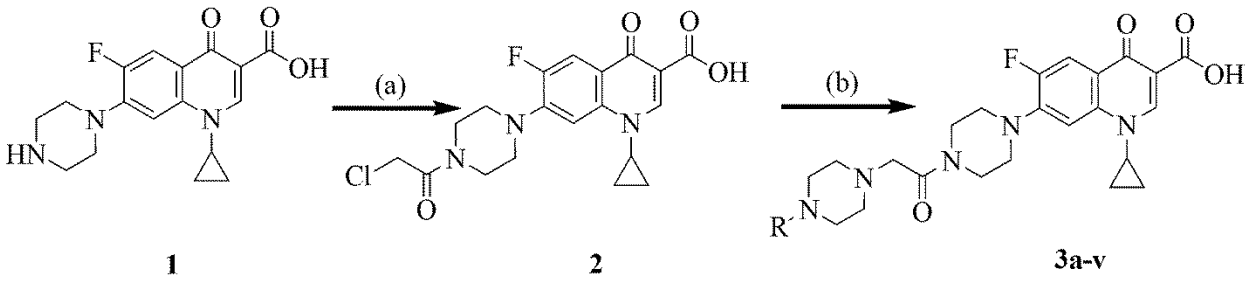
1. To design molecules based on reported anti cancer leads.
2. To synthesize the designed novel anti cancer molecules.
3. To undertake *in vitro* anti cancer screening of the synthesized compounds against various human cancer cell lines.
4. Find out amino acid interactions with molecular docking studies 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 3

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

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.

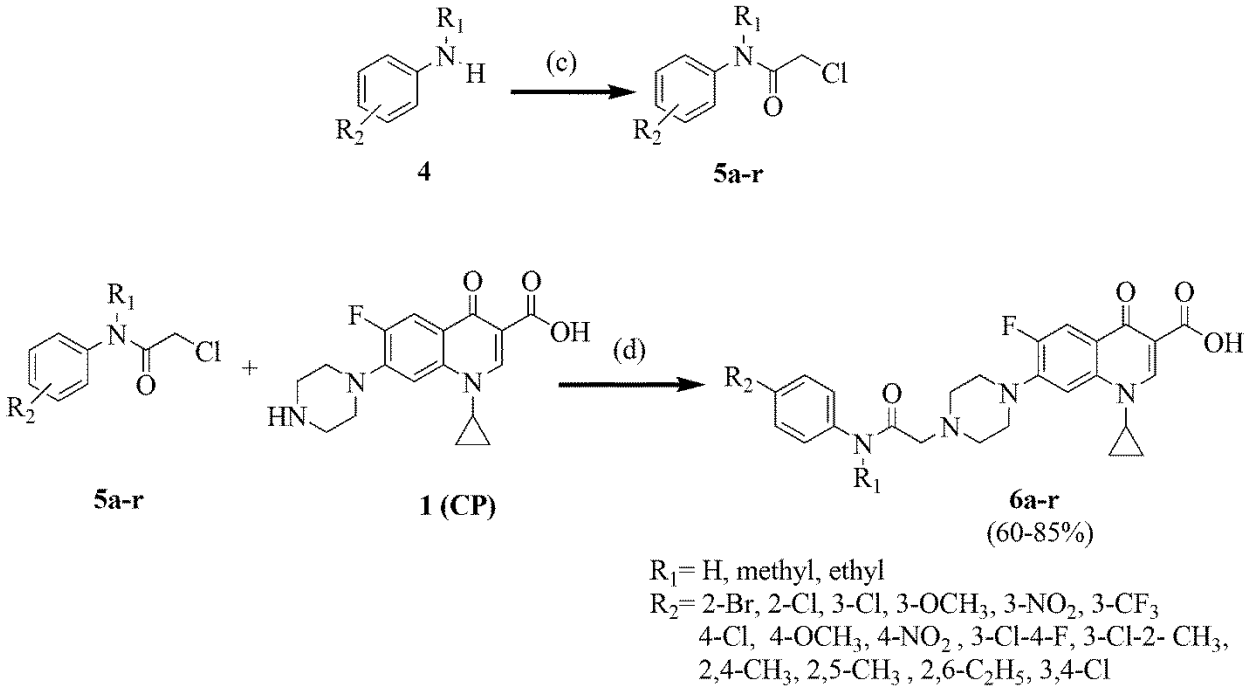
1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro quinoline-3-carboxylic acid [ciprofloxacin (CP)] is one of the broad-spectrum fluoroquinolone (FQ) antibiotics with low side effects. CP exhibited antiproliferative and apoptotic activities in several cancer cell lines such as hormone resistant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



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

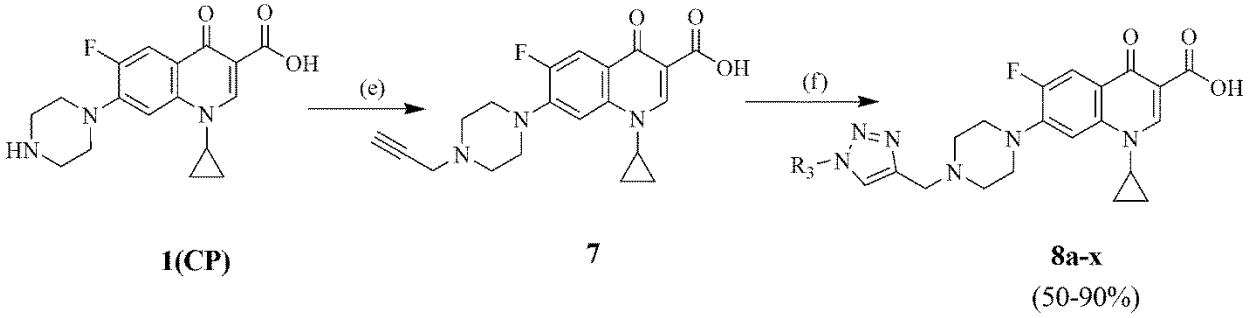
Scheme 1: Synthetic protocol utilized for the synthesis of compounds **3a-v**

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.



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



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

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.

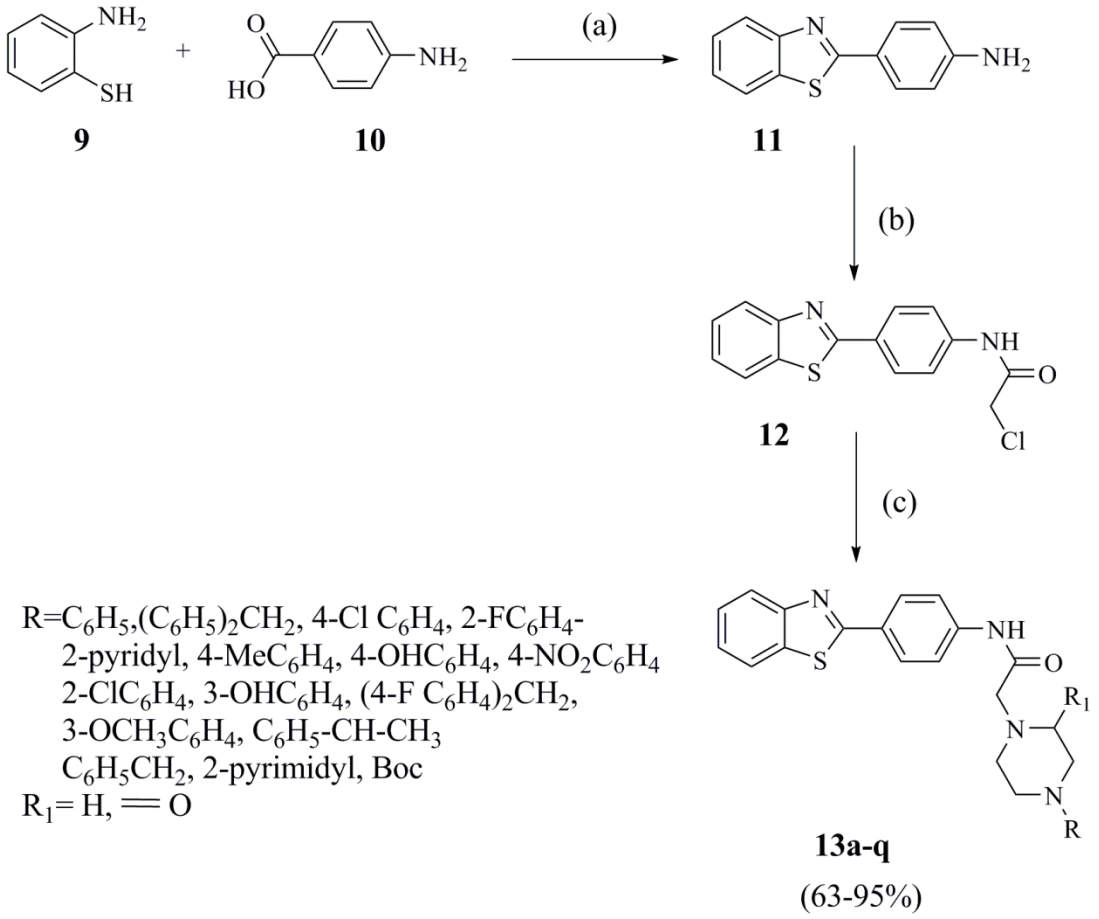
In summary in scheme 2 we conclude, electron donating substitution affects the antiproliferative activity of **CP** derivatives. These results reveal the importance of chloro, methoxy, methyl substituents at dissimilar positions and further modification on the chemical structure of **CP** derivatives 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.

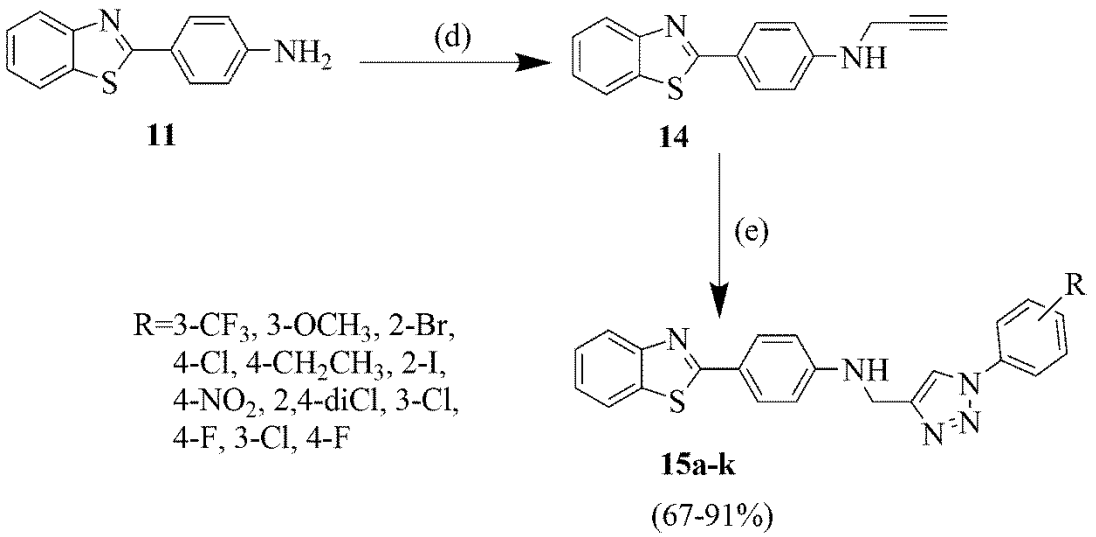
Chapter 4

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

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.



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



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

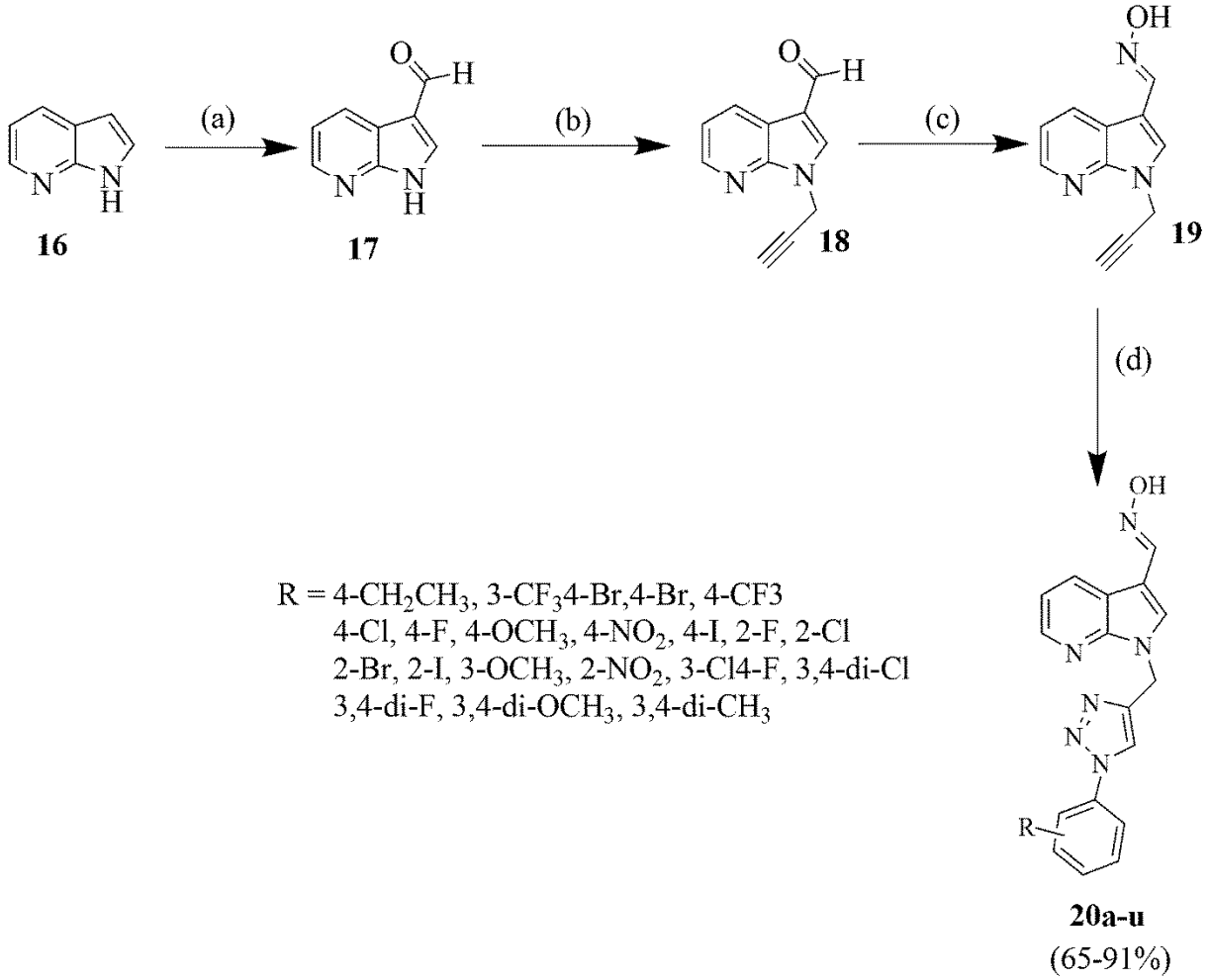
In vitro antiproliferative activity of the synthesized compounds **13a-q** and **15a-k** was carried out against three types of human cancer cell lines; A549 (lung cancer), HeLa (cervical cancer) and MDA-MB-231(breast cancer) employing sulforhodamine B (SRB) assay method.

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.

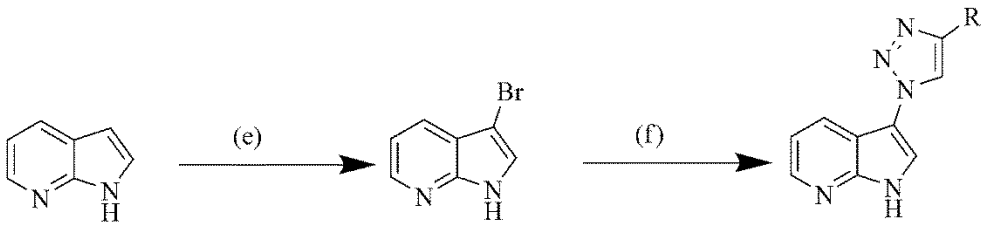
Chapter 5

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

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.



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.



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)

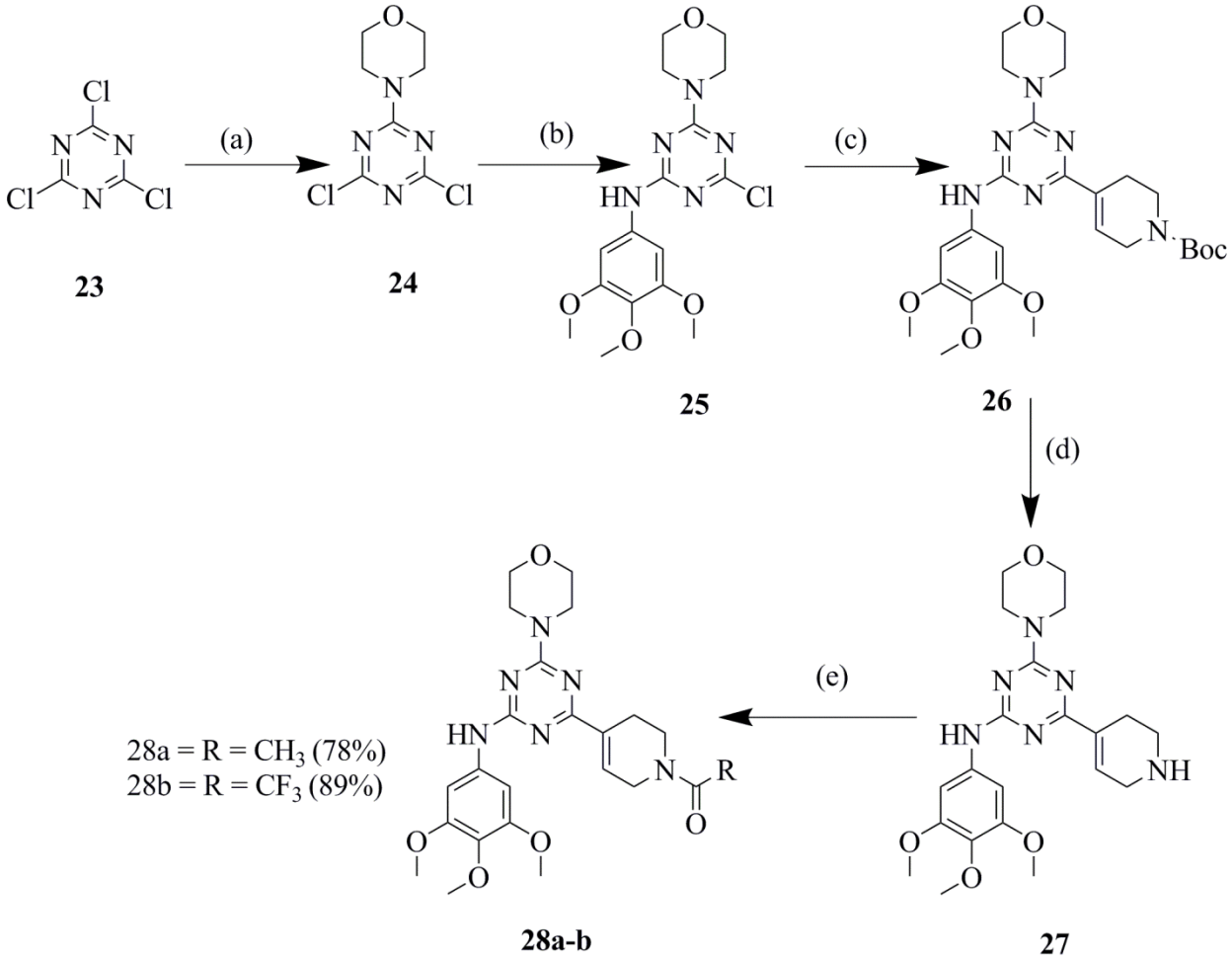
In vitro antiproliferative activity of the synthesized compounds **20a-u** and **22a-k** were evaluated against three types of human cancer cell lines; A549 (Lung cancer), HeLa (Cervical cancer) and MDA-MB-231 (Breast cancer) employing sulforhodamine B (SRB) assay method.

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.

Chapter 6

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

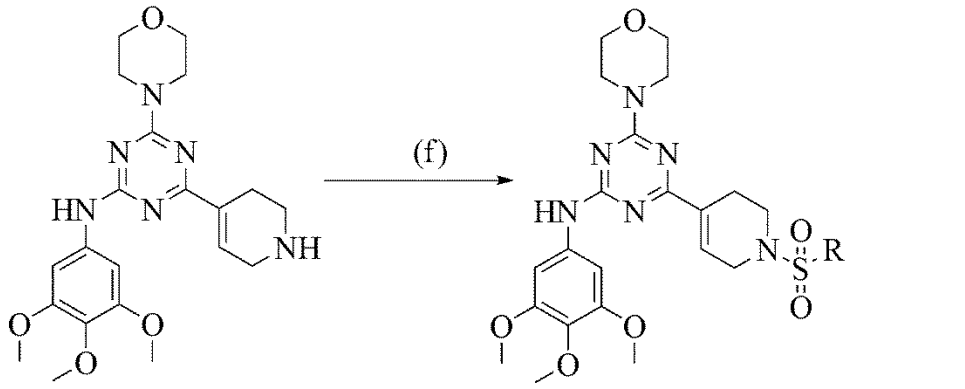
In chapter 6, series of thirty seven novel 1,3,5-triazine analogues have been synthesized, characterized and evaluated their antiproliferative activity against HeLa, HepG2, A549, and MCF-7, using sulforhodamine B assay method. Among the synthesized compounds **30j** exhibited comparable inhibitory action.



23 **24** **25** **26** **27** **28a-b**

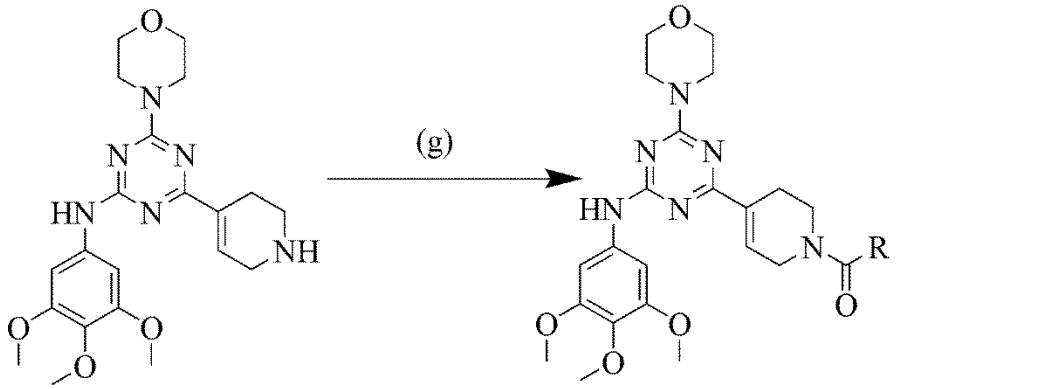
Scheme 8: Synthetic protocol to achieve the compound (**28a-b**)

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



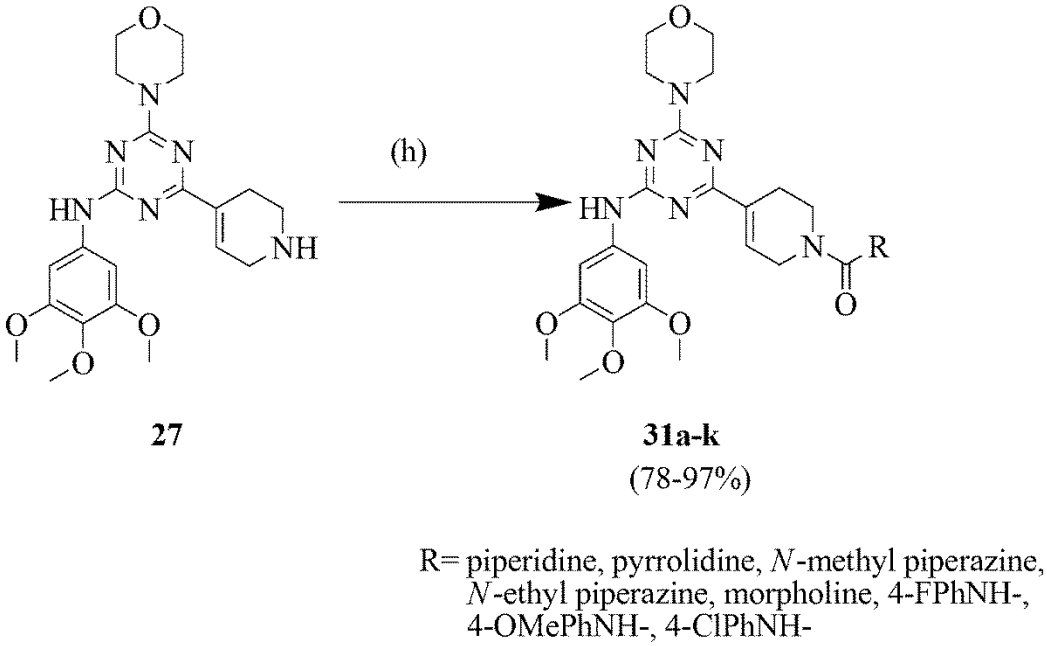
Scheme 8.1: Synthetic protocol to achieve the compound (**29a-l**)

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



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



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.

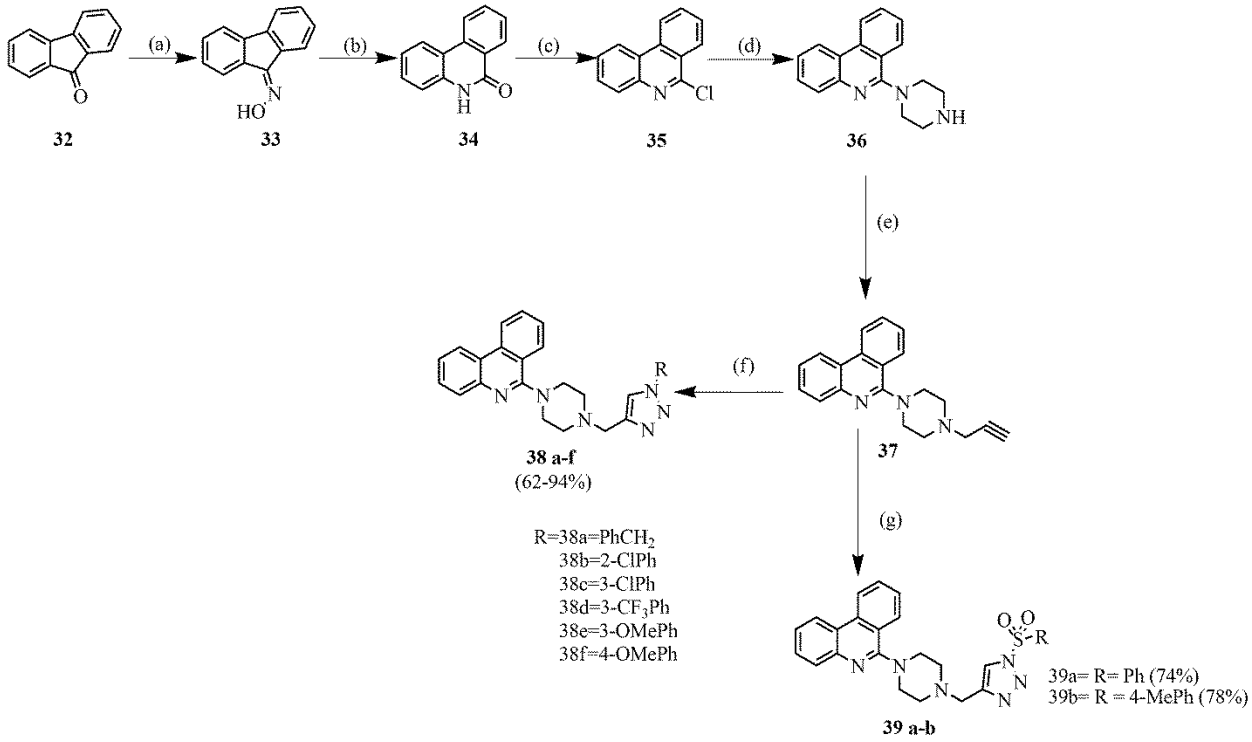
In vitro antiproliferative activity of the synthesized compounds **28a-b**, **29a-l**, **30a-l** and **31a-k** were evaluated against four types of human cancer cell lines; HeLa (Cervical cancer), HepG2 (liver carcinoma cancer), A549 (Lung cancer), and MCF 7 (Breast cancer) employing sulforhodamine B (SRB) assay method.

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.

Chapter 7

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

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.



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.

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.

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 anti-proliferative 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.