AIMS AND OBJECTIVES

3.1. Need for newer anticancer agents

Across the globe great resources have been used for prevention, diagnosis, and treatment of cancer. Cancer is the major cause of mortality in the world. Discovery and development of new anticancer agents are key focus of many pharmaceutical companies and various organizations such as National Cancer Institute (NCI) in the United States, the European Organization for Research and Treatment of Cancer (EORTC), and the British Cancer Research Campaign (CRC). The major challenge is to design new targeted drugs that will be more selective for cancer cells, and thus have lesser side effects. The biochemical aspects of cancer to target a tumor cell selectively could be explored more. Only a few compounds reach to the clinic out of a huge library. Both small and large molecular compounds continue to be tested as anticancer agents. Their exact mechanism of action is often a subject of retrospective investigation. Targeted strategy has achieved significant success, the recent developments in molecular biology and an understanding of the pharmacology of cancer at a molecular level have challenged researchers to come up with target-based drugs.

In case of EGFR TK inhibitors, currently available chemotherapy for treatment of cancer provides symptomatic relief and they fail to halt disease progression because of development of resistance. The design of drugs as EGFR TKIs has always been challenging but has helped the patients who are not responding to the earlier therapies. Therefore, searching for agents particularly effective against kinase and EGFR TK remains urgent. It is hoped that new candidates will be a big breakthrough in the treatment of carcinoma.

3.2 Objectives of the study

As has been mentioned above, the aim of the study was to design and synthesize new anticancer agents. To start the work, it was decided to use the pharmacophore based approach. In literature, various kinase and EGFR tyrosine kinase inhibitors are reported. Accordingly, pharmacophore was developed using these compounds as given below:

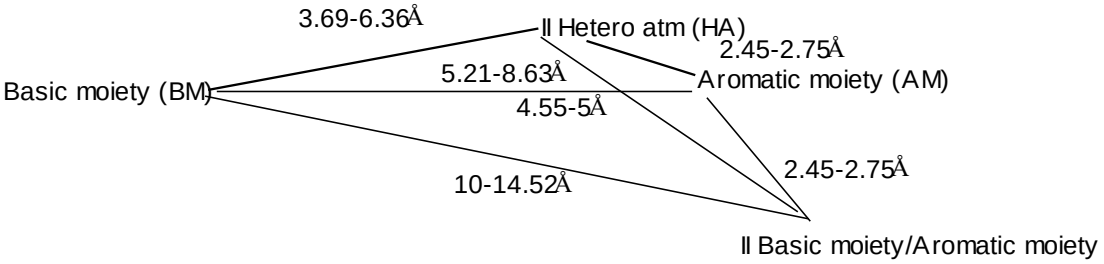


Figure 3.1: Proposed pharmacophore for kinase inhibition.

Table 3.1: Pharmacophoric distances of some representative kinase inhibitors used to develop the pharmacophore model.

Compound name (Number)	Distances in angstrom (Å)			
	BM-HA (3.69-6.36)	HA-AM (2.74-2.76)	BM-AM (6.64-8.63)	BM- IIBM /IIAM (10.60-14.52)
Vandetanib (6)	5.16	2.75	7.50	13.99
Nintedanib (16)	3.69	2.75	7.89	11.53
Gefitinib (18)	4.90	2.73	7.73	10.98
Decomitinib (20)	6.36	2.74	8.63	10.96
Canertinib (21)	4.31	2.74	6.64	11.89
Olmutinib (27)	5.59	2.75	7.86	10.60
ASP8273 (26)	4.31	2.74	7.12	11.95
WZ002 (30)	5.61	2.75	7.82	12.10
Copanlisib (31)	4.88	2.76	7.52	14.52
Salicylamide (32)	4.89	2.76	7.52	13.57

Using this pharmacophore, it was decided to work on four series (seven scaffolds) of compounds with an aim to:

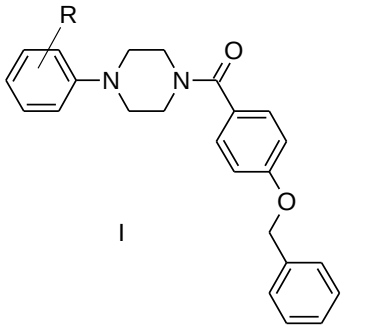
1. check the effect of removing second basic/aromatic moiety on anticancer activity (scaffold-I). However, phenyl piperazine motif was added, which is reported to have anticancer properties [51] .

2. replace anilino quinazoline scaffold present in most of the reported compounds, which is responsible for rash and diarrhea, with aromatic ring (scaffolds II-VII).

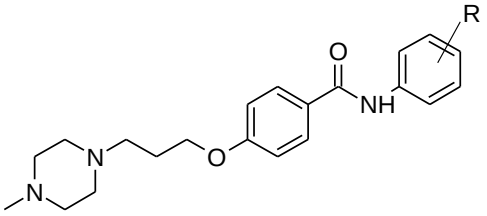
3. add amide group as it has been reported to impart kinase inhibition.

4. check the effect of substituting electron donating and electron withdrawing substituents on anticancer activity.

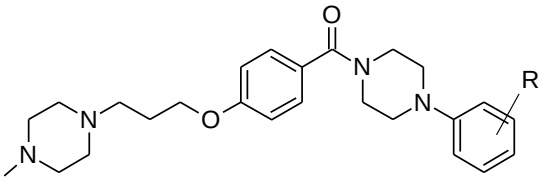
Keeping the above in mind, the scaffolds were selected and the pharmacophoric distances of these scaffolds are given in table 3.2.



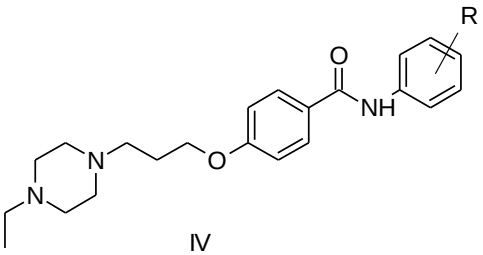
I



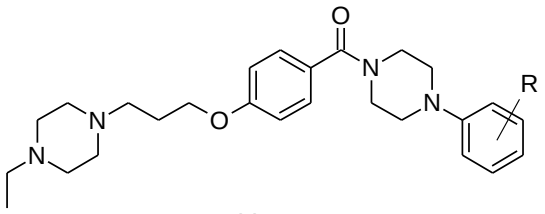
II



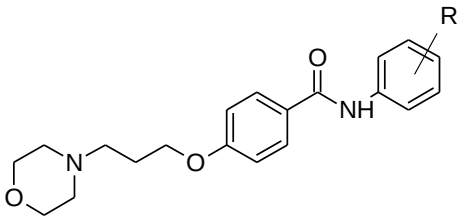
III



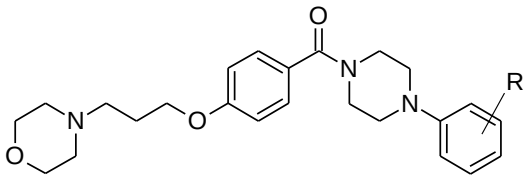
IV



V



VI



VII

Table 3.2: Pharmacophoric distances of selected scaffolds

Scaffold no.	Distances in Angstrom (Å)			
	BM-HA	HA-AM	BM-AM	BM- IIAM
I	6.48	3.69	9.91	-
II	4.88	2.73	7.52	13.95
III	4.90	2.75	7.51	13.91
IV	4.89	2.72	7.51	13.90
V	4.90	2.73	7.50	13.89
VI	4.90	2.71	7.52	13.88
VII	4.89	2.72	7.53	13.90

Although initial plan was to synthesize anticancer compounds and to study their anticancer potential in carcinoma cell lines only, later studies were planned to test the mechanistic aspects also. Since the pharmacophore used was for kinase inhibition, of which most of the compounds were EGFR TKIs, it was planned to study the binding interactions of the synthesized compounds with EGFR active site. The availability of several pdb crystal structures of EGFR made it possible. Therefore, the present work also included *in silico* studies to verify the EGFR inhibitory potential. To further validate, *in vitro* inhibition studies were also planned.

To achieve these objectives, following steps were envisaged:

Step 1: To synthesize the designed (using pharmacophoric approach) library of small molecules followed by purification and characterization.

Step 2: To perform cytotoxicity studies of synthesized compounds cell lines.

Step-3: To study the mechanistic aspects, perform docking studies for EGFR inhibition.

Step-4: As a proof-of-concept, to screen these compounds for their EGFRTK inhibitory potential using *in vitro* assay methodology.