SUMMARY AND CONCLUSION

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Cancer is one of the most dangerous diseases in today's modern world. Cancer is formed when normal cells lose their normal regulatory mechanism that control growth and multiplication. Based on tissue types, cancer may be classified as sarcoma, lymphoma, leukemia and adenoma and carcinoma. Carcinoma is the most common type of cancer and arises from epithelial cells and may invade adjacent tissue, organs and metastasizes all organs. Breast, prostate, pancreatic, lung and colon cancers are some example of carcinoma. The global burden of cancer is expected to grow to 21.7 million as new cancer cases and 13 million cancer deaths in 2030. With more than 1,300 deaths reported every day, cancer in India has become one of the major causes of death. Discovery and development of new anticancer agents are key focus of many pharmaceutical companies and various research 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 biochemical aspects of cancer to target a tumor cell selectively could be explored more. 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. Hence, it was thought to make new anticancer compounds.

To start the work, it was decided to use the information available in literature. In literature, various kinase inhibitors are reported for carcinomas. Using reported compounds, pharmacophore was designed and using this pharmacophore, it was decided to work on four series of compounds which led to seven structural scaffolds. However, there was no intention to develop kinase inhibitors specifically. Intention was to derive new scaffolds, which are not reported in literature, which could show anticancer potential.

To synthesize scaffold-I it was decided to remove the second basic /aromatic moiety to check the effect on anticancer activity. Further, instead of quinazoline nitrogen, aromatic amide group as hydrogen bond acceptor was added to all seven scaffolds. Addition of amide group gives the characteristic of second and third generation kinase anticancer compounds. All the compounds

were substituted with electron donating and electron withdrawing groups to check the effect on anticancer activity.

Although initial plan was to synthesize anticancer compounds only, later studies were planned to test the mechanistic aspects. Since the pharmacophore used was for kinase inhibition, and the cell lines used had EGFR overexpression, it was planned to study the binding interactions of the synthesized compounds with EGFR active site, and to further validate in vitro inhibition studies were also planned.

A total of sixty four compounds were synthesized. Cell line study was performed to confirm whether compounds are cytotoxic or not. In adenocarcinomic human alveolar basal epithelial cell line (Lung cancer, A-549) gefitinib showed IC_{50} value of 16.56 µM, in human colon carcinoma cell line (HCT-116 cell line) it showed IC_{50} value of 10.51 µM, whereas in human pancreatic carcinoma MIAPaCa-2 cell line, gefitinib showed IC_{50} value of 49.50 µM.

Among the compounds with scaffold I, it was observed that overall; electron withdrawing substituted compounds were devoid of cytotoxicity. In Mia PaCa-2 cell line compound A-7 showed better inhibition than gefitinib wherein it had IC_{50} value of 9.90 µM. Among scaffold II and III compounds, best IC_{50} value was of compound B-1 (6.54 µM) in HCT-116 colon cancer cell line. From compounds belonging to scaffold IV and V, in A-549 lung cancer cell line and HCT-116 colon cancer cell line, electron donating substitution showed better inhibition when compared to electron withdrawing substitution. In HCT-116 colon cancer cell line, compound C-2 resulted in better inhibition than C-3 and in disubstituted, C-4 (2,4 di-CH3) showed comparable inhibition to that of gefitinib. Among scaffold VI and VII compounds, in MIAPaCa-2 cell line, compound D-4 was the most active with IC_{50} values of 11.25 µM.

In A-549 lung cancer cell line three compounds (B-6, B-11, B-16), in HCT-116, Four compounds (B-1, B-4, B-11, C-4) and in MIAPaCa-2 pancreatic cancer cell line eighteen compounds (A-8, A-10, B-1, B-2, B-4, B-5, B6, B-7, B-10, B-11, B-12, B-14, C-1, C-2, C-16, D-4, D-14, D-14) showed better inhibition than standard drug gefitinib.

In the cytotoxicity studies, a number of compounds showed very good inhibition. Therefore, it was decided to explore the possible mechanism of action. Since the cytotoxicity was observed in A-549, HCT-116 and MiaPaCa-2 cell lines, which are well known for EGFR expression, it was

considered to check the EGFR inhibition via docking studies. Crystal structure of human EGFR (2ITO: along with ligand gefitinib) protein was used and docking was performed using Schrodinger Suite 2013. Physicochemical properties such as molecular weight, hydrogen bond donor (HBD) count and hydrogen bond acceptor count (HBA), topological polar surface area (TPSA), molar refractivity (MR), and log partition coefficient (ClogP), influence oral bioavailability were determined using Chemaxon Jchem for Excel. The toxicity risk assessment was carried using OSIRIS property explorer. For EGFR inhibition, hydrogen bonding with CYS797, ASP800 or ASP855, THR854 and ASP855 is also required and it was not observed with any of the compounds.

For EGFR inhibition, back bone hydrogen bonding with MET793 in the hinge region is required. Further hydrogen or ionic bonding with ASP800 or ASP855, THR854 and ASP855 also contributes to EGFR inhibition. From all of the synthesized compounds, many of the compounds showed hydrogen bonding with MET793 of hinge region and required hydrophobic interactions. None of the compounds, except one (D-20) resulted in more than one backbone hydrogen bonding in the hinge region.

To confirm whether the cell growth inhibition is a result of EGFR inhibition or not, EGFR assay were performed for active compounds. For studying the EGFRTK inhibitory potency of compounds, *in vitro* inhibition assay was done at BPS Biosciences, Santiago, USA. The concentration selected was 10µM as most of the compounds had IC_{50} value around 10-25µM. Hence we expected that at this concentration at least some inhibition would be seen. When the compounds were tested against EGFRTK using kit, all the compounds showed poor inhibition. The readings were not significantly different than that of vehicle control. Thus, although docking results were promising, in vitro results were not at all in concurrence.

Therefore, we conclude that the series does have potential to be explored as anticancer agents but its mechanism is not through EGFRTK inhibition.

FUTURE PERSPECTIVES

The focus of present work was mainly on generation of structurally diverse library of small molecules for anticancer agents. Based on the work done, following future studies may be planned:

1. The study has identified some structures to be cytotoxic. Using this information, several libraries can be designed from the molecules having improved activity. Modification in the active structures can be done to enhance potency and reduce failures.
2. The study has also shown that In A-549 lung cancer cell line three compounds (B-6, B-11, B-16), in HCT-116, Four compounds (B-1, B-4, B-11, C-4) and in MIAPaCa-2 pancreatic cancer cell line eighteen compounds (A-8, A-10, B-1, B-2, B-4, B-5, B6, B-7, B-10, B-11, B-12, B-14, C-1, C-2, C-16, D-4, D-14, D-14) showed better inhibition than standard drug gefitinib. Activity in other cell lines too can be explored.
3. To further prove the efficacy of these compounds, in vivo studies may be planned.
4. Since it was observed that these compounds showed poor EGFR TK inhibition, further studies such as inhibition of other kinases may be planned to find out their mechanism of action.