The thesis entitled **"Synthesis of Imidazo[2,1-*b*]thiazole and Benzo[*d*]imidazo[2,1-*b*]thiazole Conjugates as Potential Microtubule Targeting Agents"** has been divided into four chapters.

Chapter-I: General introduction of cancer and tubulin:

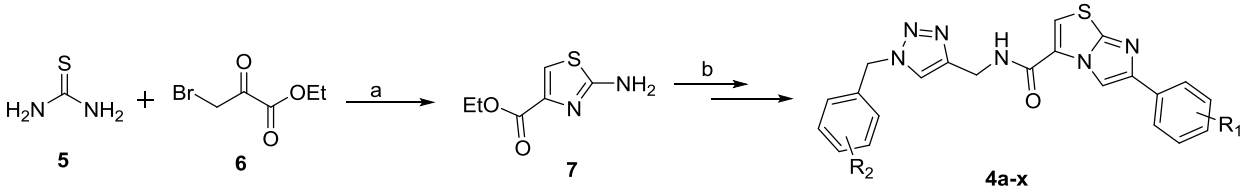
Cancer is a class of diseases or disorders characterized by uncontrolled/ abnormal division of cells and the ability of these to spread, either by direct growth into adjacent tissue, or by implantation into distant sites by *metastasis*, in which cancer cells are transported through the bloodstream or lymphatic system. Small molecules that disrupt microtubule/tubulin dynamics are used widely in cancer treatment. Hence discovery and development of novel small molecules useful in cancer chemotherapy has identified tubulin as a possible molecular target. Microtubules are one of the key structural components of the cytoskeleton in eukaryotic cells comprising of α and β-tubulin heterodimers. They play a crucial role in various cellular processes and have emerged as an attractive and viable target in the development of anticancer drugs mainly due to their indispensability in mitotic cell division. Generally, drugs that target microtubules bind to one of the three main sites of tubulin which includes the *taxane*-site for the microtubule stabilizing agents, the *vinca* domain and the colchicine domain for the destabilizing agents. The agents that interfer with the dynamic stability of the microtubules, act as spindle poisons arresting the dividing cells in G2/M phase of the cell cycle, causing mitotic catastrophy and finally leading to apoptotic cell death.

Statement of Problem:

Chapter-IIA: Design and Synthesis of Imidazo[2,1-*b*]thiazole linked Triazole Conjugates: Microtubule-Destabilizing Agents.

In recent years there is considerable interest in the development of new hybrid molecules that have been recently developed as anticancer agents were obtained by the combination of different pharmacophores. The promising biological activity exhibited by these hybrids prompted us to develop some newer hybrid molecules by linking the imidazo[2,1-*b*]thiazole with triazole scaffold with a view to enhance their anticancer activity. Thus a library of twenty four imidazo[2,1-*b*]thiazole linked triazole conjugates (**4a-x**) were designed, synthesized and evaluated for their cytotoxic potential.

Scheme:



Results and discussions: A series of new imidazo[2,1-*b*]thiazole linked triazole conjugates (**4a-x**) were synthesized and evaluated for their cytotoxic potency against HeLa, DU-145, A549, MCF-7 and HepG2 cell lines. Among them, conjugates **4g** and **4h** exhibited most cytotoxic potency effect against A549 cells with IC_{50} values of 0.92 and 0.78 µM respectively. FACS analysis revealed that these conjugates induced cell cycle arrest in G2/M phase in A549 lung cancer cells. The tubulin polymerization assay and immunofluorescence analysis showed that these conjugates effectively inhibit microtubule assembly in cell-free and cell-based (A549) experiments respectively. Moreover, the apoptosis inducing properties were evaluated by Hoechst staining, mitochondrial membrane potential and Annexin V-FITC assay. Further, western blot analysis was performed for proapoptotic protein Bax and antiapoptotic protein Bcl-2, and the results demonstrated that there was up regulation of Bax and down regulation of Bcl-2 suggesting that these compounds induce apoptosis in human lung cancer cells, A549.

Conclusion: In conclusion, a series of imidazo[2,1-*b*]thiazole linked triazole conjugates were synthesized and evaluated for their antiproliferative activity, wherein conjugates **4g** and **4h** showed significant cytotoxic activity. The FACS results revealed that these conjugates cause cell cycle arrest at G2/M phase, oreever they effectively inhibit microtubule assembly and disrupt the microtubule organization in the lung cancer cells. The molecular docking studies revealed that these conjugates bind at the colchicine site of

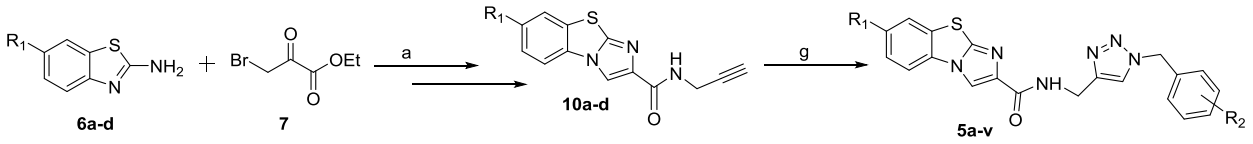
the tubulin. Further, Hoechst staining, mitochondrial membrane potential and Annexin V FITC assay suggest that they induce cell death by apoptosis. Bax and Bcl-2 results also suggest that these conjugates induce apoptosis in A549 cells. The SAR provided a useful insight that could be utilized in developing improved newer leads for the treatment of lung cancer based on such scaffolds.

Statement of Problem:

Chapter-IIB: Design and Synthesis of 1,2,3-Triazolo Linked Benzo[*d*]imidazo[2,1-*b*]thiazole Conjugates as Tubulin Polymerization Inhibitors

Nitrogen-bridge head fused heterocycles containing an imidazole and benzothiazole ring are a common structural moiety in many pharmacologically important molecules that display a wide range of activities for diverse targets. Among them, benzo[*d*]imidazo[2,1-*b*]thiazole class of compounds like 2-arylbenzo[*d*]imidazo[2,1-*b*]thiazole derivative YM-201627 was found to be orally active for the treatment of solid tumors. Another derivative AC220 was found to be highly active against FMS-like tyrosine kinase-3 (FLT3) and is in phase III clinical trials. Whereas, 1,2,3-triazoles have attracted the medicinal chemists, due to their wide range of biological activities and high metabolic stability as well as hydrogen-bonding capability with bimolecular targets. Herein we report the synthesis of 1,2,3-triazolo linked benzo[*d*]imidazo[2,1-*b*]thiazole conjugates (**5a-v**) and were evaluated for their cytotoxic potential with a view to produce promising anticancer agents.

Scheme:



Results and discussions: These newly designed 1,2,3-triazolo linked benzo[*d*]imidazo[2,1-*b*]thiazoles conjugates (**5a-v**) were synthesized and evaluated for their cytotoxic potency against the DU-145, HeLa, MCF-7, HepG2 and A549 cancer cells. Among them, conjugates like **5f** and **5k** exhibited significant antiproliferative effect against MCF-7 cells with IC_{50} values of 0.60 and 0.78 μM respectively. FACS analysis of the cell cycle demonstrated an increase in the percentage of cells in the G_2/M phase which was further authenticated by elevation of cyclin B1 protein levels. Immunocytochemistry revealed loss of intact microtubule structure in cells treated with **5f** and **5k,** and western blot analysis revealed that these conjugates accumulated more tubulin in the soluble fraction. Moreover, the conjugates caused apoptosis of the cells that was confirmed by mitochondrial membrane potential and Annexin V-FITC assay. Molecular docking studies indicated that these conjugates occupy the colchicine binding site of the tubulin protein.

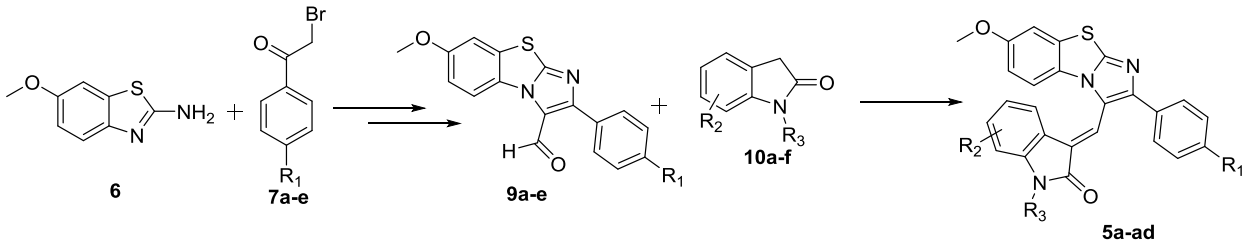
Conclusion: In the present investigation, a new class of 1,2,3-triazolo linked benzo[*d*]imidazo[2,1-*b*]thiazoles conjugates were synthesized and evaluated for their cytotoxic activity. Among them, conjugates **5f** and **5k** showed significant cytotoxic activity against the MCF cell line. The FACS analysis revealed that these conjugates cause cell cycle arrest at G_2/M phase. The presence of increased levels of the cyclin B1 protein and tubulin in the soluble fraction of cells corroborate well with the tubulin polymerization inhibition. Furthermore, they effectively inhibit microtubule assembly and disrupt the microtubule organization in the breast cancer cells. The molecular docking studies revealed that these conjugates bind at the colchicine site of the tubulin. The induction of apoptosis is associated with mitochondrial membrane potential and Annexin V FITC assay. Thus these conjugates could be considered as potential scaffolds for the development of new leads as chemotherapeutics for breast cancer.

Statement of Problem:

Chapter–IIIA: Design and synthesis of C3-Oxindole Linked Benzo[*d*]imidazo[2,1-*b*]thiazole Conjugates as Cytotoxic and Apoptotic Inducing Agents

Pharmacophore hybridization is an effective tool to design highly active new chemical entities by covalently combining two or more active pharmacophores into a single molecule. These hybrids may act on multiple targets and offer the possibility of overcoming drug resistance, and in addition, hybrids may also often show synergetic action. In view of the above considerations and inspired by the promising anticancer activity of benzo[*d*]imidazo[2,1-*b*]thiazole and oxindole, we synthesized a series of C3 oxindole linked benzo[*d*]imidazo[2,1-*b*]thiazole conjugates (**5a-ad**) by combining benzo[*d*]imidazo[2,1-*b*]thiazole with oxindoles *via* knoevenagel condensation with a view to produce promising anticancer agents.

Scheme:



Results and discussions: A series of C3 oxindole linked benzo[*d*]imidazo[2,1-*b*]thiazole conjugates (**5a-ad**) were designed, synthesized and evaluated for their cytotoxic potency against some human cancer cell lines like A549 (lung), DU-145 (prostate), MCF-7 (breast) and HT-29 (colon). Preliminary results revealed that some of these conjugate like **5b** exhibited significant antiproliferative effect against human DU-145 (prostate) cancer cells with IC_{50} values of 0.889 µM. FACS analysis of the cell cycle demonstrated an increase in the percentage of cells in the G_2/M phase. Moreover, the apoptosis inducing effect of the compound was studied using Hoechst staining, mitochondrial membrane potential, Annexin V-FITC assay. Based on these studies, **5b** have been identified as promising new molecule that have the potential to be developed as leads.

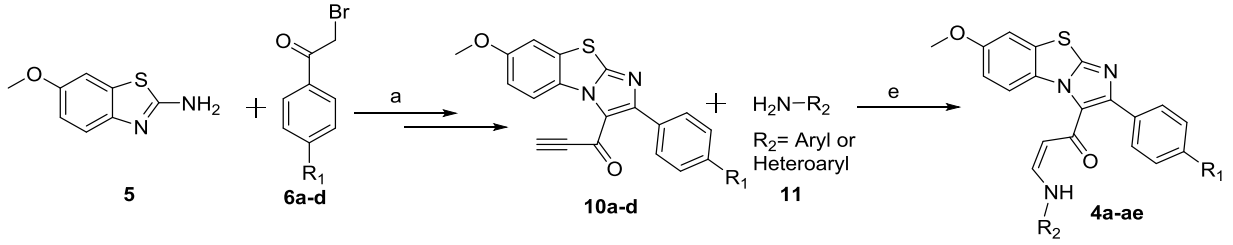
Conclusion: In summary, a new series of C3 oxindole linked benzo[*d*]imidazo[2,1-*b*]thiazole conjugates (**5a-ad**) were synthesized and screened for cytotoxic potency against four human cancer cell lines. Conjugate **5b** showed significant potency against DU-145 cells. FACS analysis indicates that this conjugate induce cell cycle arrest at G2/M phase.

Statement of Problem:

Chapter IIIB: Design and Synthesis of 3-Arylaminopropenone Linked 2-Arylbenzo[*d*]imidazo[2,1-*b*]thiazole Conjugates as Cytotoxic and Apoptotic Inducing Agents

Benzo[*d*]imidazo[2,1-*b*]thiazole scaffold containing compounds like YM-201627 and AC220 were found as potent anticancer agents. Whereas, propenones are considered as suitable linkers for the hybridization of promising compounds. Based on this background it was considered of interest to synthesize 3-arylaminopropenone linked 2-arylbenzo[*d*]imidazo[2,1-*b*]thiazole conjugates (**4a-ae**) with a view to explore their cytotoxic potential.

Scheme:



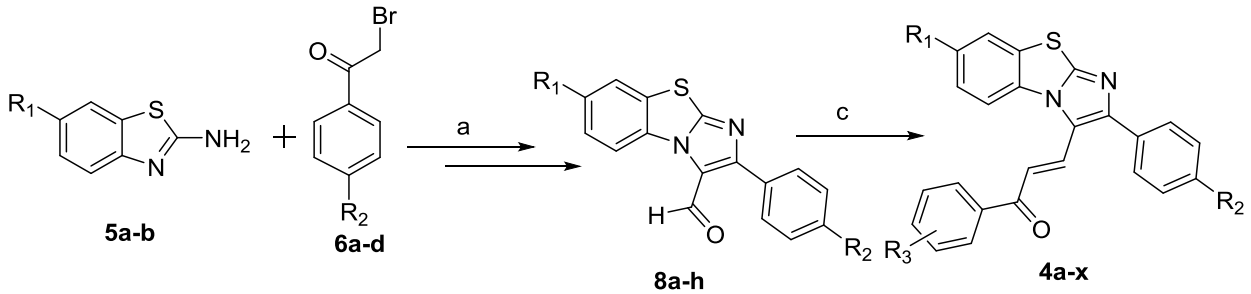
Results and discussions: A series of 3-arylaminopropenone linked 2-arylbenzo[*d*]imidazo[2,1-*b*]thiazole conjugates were synthesized and screened for their cytotoxic activity against four human cancer cell lines. Some conjugates like **4e**, **4h**, **4m** and **4u** exhibited good antiproliferative activity and the insight to their structure activity relationship was developed. Among them, conjugate **4e** showed significant potential particularly against HeLa cell line with IC_{50} value 1.6 µM. FACS analysis indicates that conjugate **4e** induce cell cycle arrest at G_1 phase. In addition, the potential mechanism of cell growth inhibition and apoptotic induction by this conjugate was investigated in HeLa cancer cells using cell-based assays, including wound healing assay and Hoechst staining. Moreover, conjugate **4e** led to the collapse of mitochondrial membrane potential (DΨm) and increased levels of reactive oxygen species (ROS) were noted.

Conclusion: In summary, a new series of 3-arylaminopropenone linked 2-arylbenzo[*d*]imidazo[2,1-*b*]thiazole conjugates were screened for their cytotoxic potency against four human cancer cell lines. Interestingly, conjugate **4e** showed significant potency against HeLa cells. FACS analysis indicates that it induces cell cycle arrest at G1 phase and also induces apoptosis in HeLa cells by using wound healing assay, Actin Filaments and Hoechst staining. It also causies collapse of DΨm and elevation of ROS production. Overall, these results demonstrate that conjugate **4e** has the potential to be developed as a lead and its further structural modification may produce promising anticancer agents for HeLa cancer cells.

Statement of Problem:

Chapter IVA: Design and Synthesis of Chalcone linked Benzo[*d*]imidazo[2,1-*b*]thiazole Conjugates as Potential Tubulin Polymerization inhibitors

Pharmacophore conjugation is an effective tool to design highly active new chemical compounds by keeping drug resistance and synergetic action. In this point of view and inspired by the promising anticancer activity of benzo[*d*]imidazo[2,1-*b*]thiazoles and chalcones, we synthesized a series of chalcone linked benzo[*d*]imidazo[2,1-*b*]thiazole conjugates (**4a-x**) with a view to produce promising cytotoxic agents.



Results and discussions: Chalcone linked benzo[*d*]imidazo[2,1-*b*]thiazole conjugates (**4a-x**) were designed, synthesized and evaluated for their cytoxic potency against some human cancer cell lines like cervical (HeLa), breast (MCF-7), liver (HepG2) and lung adenocarcinoma (A549). Preliminary results revealed that some of these conjugates like **4b** and **4c** exhibited significant antiproliferative effect against HeLa cells with IC_{50} values of 1.6 and 2.1 µM respectively. FACS analysis revealed that they arrest the cell cycle at the G_2/M phase and further authenticated by elevation of cyclin B1 protein levels. Immunocytochemistry revealed loss of intact microtubule structure in cells treated with **4b** and **4c** and western blot analysis revealed that these conjugates accumulate more tubulin in the soluble fraction. Molecular docking studies indicated that these conjugates occupy the colchicine binding site of the tubulin protein.

Conclusion: In summary, a series of chalcone linked benzo[*d*]imidazo[2,1-*b*]thiazole conjugates (**4a-x**) were screened for cytotoxic potency against four human cancer cell lines. Among them, conjugate **4b** and **4c** showed significant potency against HeLa cells. FACS analysis indicates that these conjugates induce

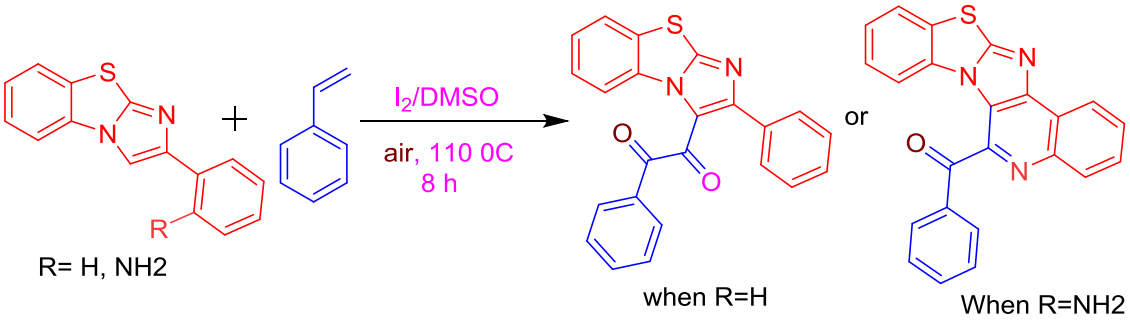
cell cycle arrest at G_2/M phase and they inhibit microtubule assembly and disrupt the microtubule organization in the HeLa cancer cells.

Statement of Problem:

Chapter IVB: Regioselective Oxidative Cross-Coupling of Benzo[*d*]imidazo[2,1-*b*]thiazoles with Styrenes: A New Route to C3-Dicarbonylation of Benzo[*d*]imidazo[2,1-*b*]thiazoles

The oxidative cross-coupling of two different C-H bonds for the construction of specific C-C bonds in the development of effective synthetic strategies to functionalize biologically important pharmacophores is an ongoing interest in organic and medicinal chemistry. 1,2-Dicarbonyl compounds are valuable starting materials and are important synthetic intermediates in the preparation of many fine chemicals that could be readily converted to many other functional groups and have become attractive targets. Therefore, development of metal-free, peroxide-free, oxidant-free and environmentally benign methods with terminal alkenes via C(sp^2)-H bond cleavage is still challenging and highly desirable for 1,2-dicarbonyl functionalization *via* C-H bond cleavage to obtained biologically active heterocyclics.

Scheme:



Results and discussions: A novel I_2 promoted, highly efficient metal-free and peroxide-free greener domino protocol for C3-dicarbonylation of benzo[*d*]imidazo[2,1-*b*]thiazoles (IBT) with styrenes has been developed *via* oxidative cleavage of C(sp^2)-H bond, followed by C3-nucleophilic attack of IBT and oxidation under mild conditions. Interestingly, under these conditions 2-(benzo[*d*]imidazo[2,1-*b*]thiazol-2-yl)aniline gave the benzo[4',5']thiazolo[2',3':2,3]imidazo[4,5-*c*]quinoline derivative *via* oxidative cleavage of C(sp^2)-H bond, followed by pictet spengler cyclization and aromatization. By using this simple method a library of C3-dicarbonylated benzo[*d*]imidazo[2,1-*b*]thiazole derivatives have been synthesized in moderate to good yields and screened for their antoproliferative activity. Among them **3l** showed significant cytotoxicity with an IC_{50} of 5.26 µM against MCF-7 cell line.

Conclusion: In conclusion, we have successfully established a highly efficient molecular I_2 mediated, metal-free, peroxide-free and greener domino approach for site selective oxidative C3-dicarbonylation of IBTs with styrene**s** *via* oxidative cleavage of C(sp^2)-H bond, followed by electron rich C3 attack of IBT and oxidation. This method proceeds under mild conditions with high regioselectivity and broad substrate scope. Furthermore, using this simple method a library of C3-dicarbonylated benzo[*d*]imidazo[2,1-*b*]thiazole derivatives have been synthesized in moderate to good yields and screened them for antiprolifirative activity. Among them **3l** showed significant cytotoxicity with an IC_{50} of 5.26 µM against MCF-7 cell line.