Chapter 5

Summary and Conclusion

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A. Coumarin-Quinoxaline Hybrids

By considering the anti-cancer potentials of coumarin and quinoxaline moieties, a series of coumarin-quinoxaline hybrids was designed and screened for their aromatase and HER2 binding properties through molecular docking approaches. Aromatase and HER2 are the promising targets associated with the breast cancer. The molecular docking studies were carried out using MOE software. Best twelve molecules were screened out on the basis of the docking scores which were compared to the reference drugs exemestane and trastuzumab. The interaction patterns of these molecules were also studies with the help of docking poses. Further *in silico* druglikeliness and ADME properties of these compounds were also evaluated using online tools SwissADME and PreADME. All the compounds followed the Lipinsiki's rule of five and revealed good absorption. Also *in silico* toxicity was predicted using Protox software which revealed the safety profile of the screened out molecules. After the *in silico* screening of the compounds, these best twelve compounds were synthesized and the structures of synthesized derivatives were evaluated by ^{1}H NMR, ^{13}C-NMR and HRMS. The synthesized coumarin-quinoxaline hybrids were then subjected to their antiproliferative potentials against various cancer cell lines using the MTT assay. The antiproliferative evaluation was done against two breast cancer cell lines, liver cancer cell lines and lung cancer cell lines. Compound RB17 and RB86 were found the most potent compounds among the series against all the cell lines with IC_{50} values ranging between 3.8-6.52 µM and 7.82-18.6 µM respectively whereas the cell viability was found 51-54% and 52-57% respectively which was almost equal to the reference drugs. Other molecules revealed good to moderate activity. Compounds RB17 and RB86 were also tested for normal cell toxicity against normal lung fibroblat cells WI-38. The IC50 values against these cell lines were found to be very high 178.36 and 198.36 µM respectively which was even higher than the reference drugs. This indicated that the molecules are safer for normal healthy cells.

B. Coumarin-Dihydropyrimidinone Hybrids

Several reports in literature have evidenced the anticancer potentials of coumarin and dihydropyrimidinone scaffolds. By following the rationale design approach, we have designed a library of 90 coumarin-dihydropyrimidinone hybrid molecules. The

designed molecules were then subjected to molecular docking studies against the aromatase and docking scores were obtained. The best twelve compounds with maximum docking scores were then selected for further exploration. The binding patterns were also studies with the help of docking poses and compared to the reference drug exemestane. Further *in silico* druglikeliness and ADME properties of these compounds were also evaluated using online tools SwissADME and PreADME. All the compounds followed the Lipinsiki's rule of five and revealed good absorption. Also *in silico* toxicity was predicted using Protox software which revealed the safety profile of the screened out molecules. After the *in silico* screening of the compounds, these best twelve compounds were synthesized and the synthetic protocol was optimized after making several trials by varying the concentrations of the reactants and various recation conditions. The structures of synthesized derivatives were evaluated by ^{1}H NMR, ^{13}C-NMR and HRMS. Further, the synthesized molecules were evaluated for antiproliferative activity against four cancer cell lines: breast cancer (MCF7 and T47D), liver cancer (HepG2) and lung cancer cell lines (A549). Compounds CD8, CD10 and CD28 were found the most potent among all evaluated compounds. Compounds CD28 revealed excellent potency against Breast cancer cell lines with IC_{50} values of 8.82 and 13.82 μM whereas compound CD10 revealed excellent activity against liver and lung cancer cell lines with IC_{50} values of 5.22 and 3.68 μM respectively. The obtained values for these most potent compounds were almost equivalent to the reference drugs exemestane and doxorubicin. The evaluated compounds also revealed good cell viability results within the range of 51 to 70%. Compounds CD10 and CD28 were also tested for normal cell toxicity against normal lung fibroblat cells WI-38. The IC50 values against these cell lines were found to be very high 188.42 and 144.42 μM respectively which was even higher than the reference drugs. This indicated that the molecules are safer for normal healthy cells.

C. Coumarin-Dihydropyridine Hybrids

Coumarin scaffold being a well established anticancer scaffold, Aromatase inhibitor and least toxic to the organ systems is a promising motif in drug development against cancer. Similarly dihydropyridine has also excellent anticancer as well as multidrug resistance reversal properties. By keeping in view these characteristics of the two motifs, a series of coumarin-dihydropyridine hybrids was designed to exploit their

anticancer potentials. A library of 75 compounds was designed and screened through molecular docking studies against Aromatase enzyme which a potential target in breast cancer. Among 75, twelve compounds who displayed best docking scores were selected and evaluated for the binding patterns with the Aromatase by studying the docking poses. The orientations were compared with the reference drug exemestane. Further these 12 molecules predicted to be anticancer were evaluated for *in silico* drug likeliness properties and *in silico* ADME studies. The compounds showed only one violation from the Lipinski's rule of five which was higher molecular weight of the designed molecules (above 500). The compounds showed good blood brain barrier penetration and absorption across intestine. Also *in silico* toxicity was predicted using Protox software which revealed the safety profile of the screened out molecules. After the *in silico* screening of the compounds, these best twelve compounds were synthesized and the synthetic protocol was optimized after making several trials by varying the concentrations of the reactants and various recation conditions. The structures of synthesized derivatives were evaluated by ^{1}H NMR, ^{13}C-NMR and HRMS. Further, the synthesized coumarin-dihydropyridine hybrids were evaluated for antiproliferative activity against four cancer cell lines: breast cancer (MCF7 and T47D), liver cancer (HepG2) and lung cancer cell lines (A549). Compounds DP20, DP28 and DP32 were found the most potent among the evaluated compounds. Compounds DP28 and DP32 revealed excellent potency against all the four cell lines with IC_{50} value ranging between 3.82-15.02 μM which was equivalent to the reference drugs exemestane and doxorubicin. Compound DP20 excellent potency against liver and lung cancer cell lines with IC_{50} values of 8.26 and 7.48 μM respectively in comparison to the reference drug doxorubicin (IC_{50}=5.08 and 3.36 μM). The evaluated compounds also revealed good cell viability results within the range of 51 to 68%. Compounds DP28 and DP32 were also tested for normal cell toxicity against normal lung fibroblat cells WI-38. The IC50 values against these cell lines were found to be very high 118.61 and 121.42 μM respectively which was even higher than the reference drugs. This indicated that the molecules are safer for normal healthy cells.

The overall results for all the three series of compounds evidenced that the compounds have significant anticancer activities where the anti breast cancer effect can be attributed due to the inhibition of aromatase and HER2 enzymes. Also it was

evident that the different substituents made varying contribution towards the anticancer activity. Therefore the molecules can be considered as promising leads for further drug development and exploration against cancer.