ABSTRACT

Background: Cancer is one of the prominent causes of death in the world and based on World Health Organization (WHO) report, more than 13 million cancers death will happen in 2030. Breast cancer is one of the most frequent and insidious cancer in women. It impacts about 2.1 million women per year and is one of the most lethal ailments in women. It has been estimated that about 66% postmenopausal women suffering from breast cancer are associated with estrogen dependent breast cancer. Estrogen binds to the estrogen receptors (present in mammary glands) and promotes the tumour growth in female breasts. Estrogens cause enhancement of proliferation of breast epithelial cells and estrogen dependent mammary carcinoma cells and secrete various growth factors. It has been also reported that aromatase activity is maximum in or near the tumor sites in breasts. Aromatase enzyme acts on the androgens and produce the most potent endogenous estrogen estradiol. Although many efforts for treatment of cancer diseases have been carried out and much progress have been eventuated from diagnosis to treatment of cancer, but some of cancer patients do not respond to therapy or recurrence subsequent initial response. Nevertheless, chemotherapy is a basic approach for the treatment of cancer diseases. One of the most important obstacles in chemotherapy is drug resistance to many anticancer agents. Drug-induced toxicities followed by administrating high doses of chemotherapeutic agents to overcome drug resistance were arisen. Accordingly, the discovery of new anticancer agent with promising activity and high therapeutic index is an urgent need. Many researchers have explored various heterocyclic compounds to get maximum therapeutic efficacy from them. With a vision of novel drug design, the concept of molecular hybridisation is a significant approach which involves combination of two structurally diverse motifs in a single molecule with excellent therapeutic potential. It is well evident from literature that coumarin scaffold possesses significant anticancer activity and also has aromatase inhibition potential. Coumarin and its derivatives have been found to exhibit very rare nephrotoxicity, hepatotoxicity, cardiotoxicity, dermal toxicity and other side effects. Coumarin has diverse pharmacological activities like anticoagulant, anti-HIV, antibacterial, antioxidant, antihypertensive, anti-tubercular, anticonvulsant, antifungal, anti-hyperglycemic, scavenging of reactive oxygen species (ROS), anti-inflammatory, and anticancer. It has been reported that therapeutic application of coumarins depends on

the nature of the groups present and their pattern of substitution on the basic nucleus. Coumarin scaffold possesses various sites for substitution or attachment to other scaffolds. In the presented work we have fused various potent nitrogen containing scaffolds such as dihydropyridine, dihydropyrimidinone and quinoxaline with coumarin nucleus to form hybrid molecules and explored their cytotoxic potentials.

Methods: Three types of coumarin fused/tethered nitrogen containing heterocyclic hybrid molecules were designed namely: coumarin-quinoxaline hybrids (RB1-RB90), coumarin-dihydropyrimidinone hybrids (CD1-CD90) and coumarin-dihydropyridine hybrids (DP1-DP75) were designed and subjected to molecular docking studies using the MOE software. The docking was carried out for coumarin-quinoxaline derivatives against against aromatase (PDB Id: 3S7S) and HER2 (PDO Id: 3WSQ); whereas coumarin-dihydropyrimidinone and coumarin-dihydropyridine derivatives were docked in the binding pocket of aromatase only. The twelve compounds from each series with best docking stores and binding patterns were screened out and were further tested for *in silico* drug likeliness, ADME and toxicity prediction studies using SwissADME, preADMET and PROTOX online tools. Further, these compounds were synthesized by using suitable synthetic protocols and their structures were characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometric techniques. The synthesized molecules were then evaluated for their antiproliferative potentials against breast cancer cell lines (MCF7 and T47D), liver cancer cell lines (HepG2) and lung cancer cell lines (A549) utilizing standard MTT assay procedures. MIC values and % cell viabilities were determined using exemestane, trastuzumab and doxorubicin as reference drugs. The most potent compounds among the three series were also evaluated for normal cell toxicity on human lung fibroblast cell lines WI-38. All the graphs and statistical analysis of data were performed using Graph Pad Prism 7.4 software.

Results: Docking studies revealed that the screened out molecules from each series were well occupied in the cavities of aromatase and HER2. The prominent interactions found were hydrogen bonding interactions, pie-pie interactions and arene-cation interactions at very short distances ranging between 1.2-4.5 Å. The docking protocol was validated and RMSD values were found less than 0.5. *In silico* drug likeliness prediction studies showed that coumarin-quinoxaline and coumarin-dihydropyrimidinone hybrids did not show any violation from Lipinski's rule of five and revealed good oral absorption. One violation from molecular weight was

observed in case of coumarin-dihydropyridine derivatives. All the compounds from the three series showed acceptable ADME properties with good intestinal absorption. The *in silico* toxicity prediction studies showed that the compounds were least toxic with a high predicted lethal dose greater than 900mg/kg body weight. Further the antiproliferative potentials of compound were also observed using MTT assays. Among coumarin-quinoxaline hybrids, 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. Among coumarin-dihydropyrimidinone hybrids, 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. Among coumarin-dihydropyridine hybrids, 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 % cell viability for all the compounds from the three series was found between 51-70%. The most potent compounds were further tested for toxicity on normal human lung fibroblast cell lines WI-38 and were found safer.

Conclusion: 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.