Abstract

Breast cancer continues to be a major health concern for women globally, ranking as the second leading cause of cancer-related deaths among females. Despite advances in diagnosis and available treatments, resistance to current therapies presents a significant challenge, underlining the need for novel and more effective treatment strategies. High-risk mutations and their prognostic relevance further complicate management and outcomes in breast cancer patients. In this context, sirtuins, which belong to the class III family of histone deacetylases, have gained substantial attention due to their regulatory role in multiple cellular processes including metabolism, stress resistance, and apoptosis. Given their involvement in tumor survival and therapy resistance, sirtuins, particularly SIRT1–3, have become attractive molecular targets for cancer intervention. Parallel to this, naturally occurring phytoestrogens, which mimic estrogen in both structure and function, have shown promising anticancer activity, making them potential candidates for therapeutic development.

In our study, we examined the potential of phytoestrogens as sirtuin inhibitors, focusing specifically on their molecular interactions and functional outcomes in breast cancer cells. Using molecular docking techniques, we screened various phytoestrogens for their binding affinity with sirtuin proteins. Among the candidates, coumestrol exhibited the highest binding energy and strongest interactions with SIRT1, SIRT2, and SIRT3. Molecular dynamics simulations further validated the stability of these ligand-protein complexes, showing strong and consistent binding at the active sites of the sirtuin proteins. To validate these computational findings, we performed several in vitro assays, including cell proliferation and colony formation assays, using two breast cancer cell lines: MCF-7 (hormone receptor-positive) and MDAMB-231 (triple-negative).

Treatment with coumestrol significantly reduced both cell proliferation and the number of colonies formed in these cell lines, indicating a robust antiproliferative effect.

To explore the mechanisms underlying coumestrol anticancer activity, we further conducted flow cytometric analysis, which demonstrated an increase in intracellular reactive oxygen species (ROS) levels in treated cells, suggesting that oxidative stress may contribute to its cytotoxic effects. Additionally, western blot analysis revealed a marked downregulation of SIRT1 expression following coumestrol treatment in both breast cancer cell lines. This downregulation may directly correlate with the increased ROS production and impaired cellular homeostasis observed in treated cells. These findings point to sirtuin inhibition as a central mechanism through which coumestrol exerts its anticancer effects. The dual impact of oxidative stress induction and SIRT1 suppression highlights the potential of coumestrol to disrupt cancer cell survival pathways at multiple levels.

In addition to exploring sirtuin inhibition, we aimed to understand the metabolic changes induced by coumestrol and doxorubicin treatments. For this purpose, a gas chromatography-mass spectrometry (GC-MS)-based metabolomics approach was employed to evaluate alterations in metabolic profiles in MCF-7 and MDAMB-231 cell lines following treatment. In total, 66 metabolites were identified, with notable differences in their abundance patterns between treated and control samples. Specifically, 23 metabolites were significantly altered in MCF-7 cells, while forty-three showed significant changes in MDAMB-231 cells. Among the altered metabolites, key compounds such as cholesterol, palmitic acid, and 1-monooleoylglycerol were affected, pointing to disruptions in fatty acid and cholesterol metabolism. Furthermore, amino acid

metabolism was also impaired, indicating a broader reprogramming of metabolic pathways in response to treatment.

Functional enrichment analysis supported the observation that both coumestrol and doxorubicin impact core metabolic pathways involved in energy production and biosynthesis. The alteration of amino acid and lipid metabolism is particularly important in cancer cells, which rely heavily on these pathways for rapid proliferation and survival. The metabolic perturbations induced by these treatments could thus play a synergistic role in inhibiting tumor growth and enhancing the overall therapeutic efficacy. These findings underscore the multifaceted effects of coumestrol and doxorubicin and provide an initial framework for understanding their potential combinatorial use in breast cancer treatment. The integration of metabolomics with molecular biology approaches offers a comprehensive perspective on how these agents reprogram cancer cell metabolism and viability.

Further expanding our investigation into sirtuin-targeted therapies, we explored the anticancer potential of synthetic small molecules belonging to the 2-(diarylalkyl)aminobenzothiazole class. Two derivatives, namely 7ab [1-((6-chlorobenzo[d]thiazol-2-ylamino)(3,4-dichlorophenyl)methyl)naphthalen-2-ol] and 7ba [1-((6-chlorobenzo[d]thiazol-2-ylamino)(4-bromophenyl)methyl)naphthalen-2-ol], were tested on MCF-7 breast cancer cells. Both compounds significantly inhibited cell proliferation in a dose-dependent manner, with IC_{50} values of 11.4 µM and 9.6 µM for 7ab and 7ba respectively. Docking studies and molecular dynamics simulations confirmed their high binding affinity to the SIRT1 protein. In vitro assays validated these findings, showing a decrease in SIRT1 protein expression levels and an increase in the

acetylation of p53, a known SIRT1 substrate involved in cell cycle regulation and apoptosis.

The increase in acetylated p53 levels indicates that SIRT1 inhibition by these compounds may activate apoptotic pathways. Moreover, treated cells displayed markers of autophagy, such as increased LC3-II expression and the formation of cytoplasmic vacuoles, suggesting that the compounds induce both apoptotic and autophagic cell death. These dual pathways of cell death contribute to the robust antiproliferative activity observed with the 2-(diarylalkyl)aminobenzothiazole derivatives. The ability of these molecules to target SIRT1 and simultaneously influence multiple death pathways underscores their potential as multifunctional therapeutic agents. This work not only supports the rationale for targeting sirtuins in breast cancer but also introduces new chemical scaffolds for further drug development and optimization.

In conclusion, this comprehensive study illustrates the promising role of both natural and synthetic compounds in targeting sirtuin proteins for breast cancer treatment. Coumestrol, a plant-derived phytoestrogen, exhibits significant SIRT1 inhibitory activity and induces oxidative stress and metabolic reprogramming in breast cancer cells. Similarly, novel benzothiazole-based derivatives effectively suppress breast cancer cell proliferation by inhibiting SIRT1 and triggering apoptotic and autophagic cell death. The integration of computational modelling, in vitro validation, and metabolomic profiling offers a robust framework for understanding the therapeutic impact of these compounds. These findings lay the groundwork for future investigations into sirtuin-targeted therapies and open new avenues for combination treatments in breast cancer management.