Flexible Etherified and Esterified Triphenylethylene Derivatives and Their Evaluation on ER positive and Triple Negative Breast Cancer Cell Lines

Aya Hassan1, Janette Weber2, Günter Vollmer2 Ashraf Abadi2, Nermin S. Ahmed1

1Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt 2Faculty of Biology, Institute of Zoology, Technische Universität Dresden, 01062 Dresden, Germany.

Abstract

Tamoxifen (TAM) is a selective estrogen receptor modulator (SERM). It is currently the endocrine treatment of choice for the seven enzymes that convert estrogen to active metabolites and is widely used prophylactically for women with high risk of breast cancer. TAM is majorly metabolized by to more potent 4-hydroxytamoxifen and endoxifen via CYP2D6 and CYP3A4/5 enzymes. CYP2D6 is a polymorphic enzyme and has around 63 alleles; this remarkably affects the clinical outcome of tamoxifen treatment. Herein we report novel TAM analogues that are hydrolyzed via esterases to avoid the genetic polymorphism of the CYP2D6. The novel compounds bear an enhanced solubility and bioavailability, via insertion of a methylene group between ring A and the ethylene backbone. Ring A bears a para methoxy substituent whereas ring B bears different alkoyamino side chains. In series 1, ring C bears either a propionate or a decanooate ester. In series 2, Ring B and C bears homo diakoxamino groups. Compound VII bearing an OH group on ring C showed highest relative anti-estrogenic activity of 0.32 in presence of 1 nM estradiol (E2). Compound VI bearing an OH group on ring C showed highest growth inhibitory activity on MCF-7 cells (GI50 = 0.15 µM) which is ten-fold more potent than TAM (GI50 = 1.58 µM) whereas it showed growth inhibitory activity on MDA-MB-231 cells (GI50 = 1.71 µM) which is five-fold more potent than TAM (GI50 = 6.31 µM). Compound XIII was the most potent among homo diaminoalkoxy derivatives (GI50 = 0.44) on both MCF-7 and MDA-MB-231 cell lines, respectively. It showed no estrogen receptor alpha (ERα) anti-estrogenic activity in yeast estrogen screen assay (YES). Furthermore, the COMAPRE analysis using NCI-60 cancer cell lines suggested that it has different molecular modes of action compared to some of the current anti-cancer drugs including tamoxifen, highest correlation was observed with tamoxifen followed by a reported autophagy inducer N-[6-Chloro-2-methoxyacridin-9-yl]-N′-N′-diethylbutane-1,4-diamine. These results indicate that compound XIII is an interesting candidate for novel anti-cancer agents with unique modes of action.

Introduction

Tamoxifen is a revolutionary drug in medical oncology that has saved many lives over the past fifty years. The story of development of tamoxifen is perceived as a case study in pharmaceutical innovation. A drug that started as a failure contraceptive pill ended up as a blockbuster in the field of breast cancer treatment.(1) Tamoxifen is a prodrug; it requires the intrinsic metabolizing enzymes (CYP2D6 and CYP3A4) to be converted to the more anti-estrogenic metabolites, 4-hydroxytamoxifen and endoxifen.(2) CYP3A4/5 and CYP2D6 are major isozymes involved in tamoxifen metabolism and are known to display several polymorphisms that may lead to different enzyme activities and personal variation in therapeutic effects. 14 Despite being a key player in the treatment of ER-positive breast cancer and a chemopreventive agent in women with high risk for breast cancer. The most challenging issue with tamoxifen use is the development of resistance in patients who were initially responsive to tamoxifen. Although the molecular mechanisms of resistance to tamoxifen remains vague, various mechanisms have been proposed. Some of those mechanisms involved differential metabolic activation of tamoxifen, loss of ER function/ expression, alterations in crosstalk between ER and growth factor- mediated signaling pathways, the presence of ER-negative cancer stem cells, and dynamic responses to oxidative stress.(3) Many approaches were studied to overcome tamoxifen resistance. For instance, our research group worked on development of novel analogues that can bypass CYP2D6 metabolism, these analogues were metabolized via esterases.(4,5)

Herein we report the design and synthesis of twenty-four novel compounds that are designed to bypass CYP2D6. A second series is designed to work in a non ER-dependent manner in a similar fashion to raloxifene. The analogues are designed to endure an element of flexibility to the rigid triphenylethylene backbone of tamoxifen. Ring A bears a para methoxy substituent, the effect of this electron donating, hydrophobic substituent on activity is investigated. Ring B bears different aminooalkoxy side chain, an essential feature for anti-estrogenic activity of tamoxifen analogues. Ring C bears a metabolically labile ester group, both a small and long chain propyl and decyl esters are prepared. In series 2, ring B and ring C bears identical aminooalkoxyl.

All synthesized compounds were tested for their relative activity in β-galactosidase yeast estrogen screen (YES) assay. All compounds were screened by the National Cancer Institute (NCI) for in vitro antitumor activity against 60 human tumor cell lines. Additionally, we used COMAPRE algorithm enabled us to investigate potentially unique profile of action compared to currently anti-cancer drugs.

Results and Discussion

All compounds were investigated for their anti-estrogenic activity using Yeast Estrogen Screening (YES assay). Tamoxifen and 4-OH-TAM were used as controls.

- Compounds II, III, VII are the most potent anti-estrogenic analogues, they showed relative β galactosidase activity =0.48, 0.42, 0.18, respectively.
- Compounds VIII-XIII are bis-alkoxyamino derivatives that showed no anti-estrogenic activity.
- Converting the hydroxyl group on ring C to decanoate or propionate ester group led to compounds that lack anti-estrogenic activity (XIV-XVII).
- Among all hydroxylated analogues, only compound VI showed a mean growth inhibition of >100 % despite it lacks anti-estrogenic activity.
- Compound VI showed GI50 0.15 µM on MCF-7 cell lines, this is ten-fold more active than TAM (NSC-180973) GI50 1.58 µM.
- Compound VI showed GI50 values of 1.71, 1.69, 1.54 , and 1.69 µM on TNBC namely MDA-MB-231/ATCC, MDA-MB-468, BT-549 and HS 5787.

Conclusions

- Results of COMAPRE analysis showed that all tested compounds have activity which is moderately correlated to TAM (0.51-0.63)
- The novel analogues are active against ER+ breast cancer and other types of malignancies.
- The low to moderate correlation with the seed compounds suggest that the novel analogues are examples of a novel class of anti-cancer drug however; this needs to be verified by biological testing in the future.

References

1-Quirk VM. Front Pharmacol. 2017 Sep 12;8:620.
2-Jordan VC. Steroids. 2007 Nov;72(13):829-842.