SAR Analysis of PKCβ1 Inhibition by Selective Estrogen Receptor Modulators (SERMs)

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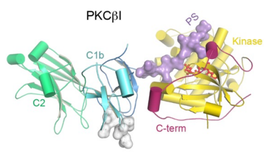
Abstract

Protein kinases C (PKCs) participate in diverse cellular pathways including regulating cell growth. Z-Tamoxifen (Z-TAM) and its active metabolite Z-Endoxifen (Z-ENDX), both being selective estrogen receptor modulators (SERMs), have been shown to be inhibitors of protein kinase C beta 1 (PKCβ1), an isoform of the PKC family. However, Z-TAM has been previously indicated to be a less potent PKC inhibitor than Z-ENDX.[1] Biochemical data from our lab has informed that Z-ENDX interact with both C- and N- domains of PKCβ1 in a non-competitive way with ATP, but the mechanistic insights into its binding are unknown.

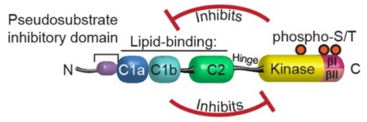
In this study, we perform structural activity relationships (SAR) analysis to compare inhibition of Z-ENDX and its derivatives and metabolites on PKCβ1. We determined IC50 values from a variety of ENDX-related SERMs with varying backbone configurations and functional groups. Overall, our data identify important features of SERMs structures for inhibiting PKCβ1, which is an important step in defining the molecular mechanism of how they bind and allosterically regulate PKCs. In future experiments, we aim to modify the structures of SERMs to increase specificity and potency for PKCs, which may lead to better anti-cancer therapies.

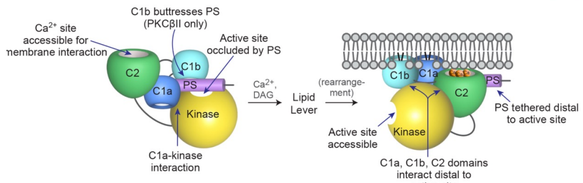
Protein Kinase C β1 Isoform



Crystal structure of PKCβ1. PKCβ1 contains 4 conserved domains shared with other isoforms: Pseudosubstrate (PS), C1a/C1b (Lipid-binding site), C2 (Ca^{2+}-binding site), and kinase domain (ATP-binding site), which phosphorylates the Ser/Thr residues of substrates. Altered activity of PKCβ1 may result in cancer and neurodegenerative diseases.[2]

Autoinhibition of PKCβ1. In the absence of lipids/Ca^{2+}, PS and lipid-binding domains inhibit kinase domain to form a closed state.

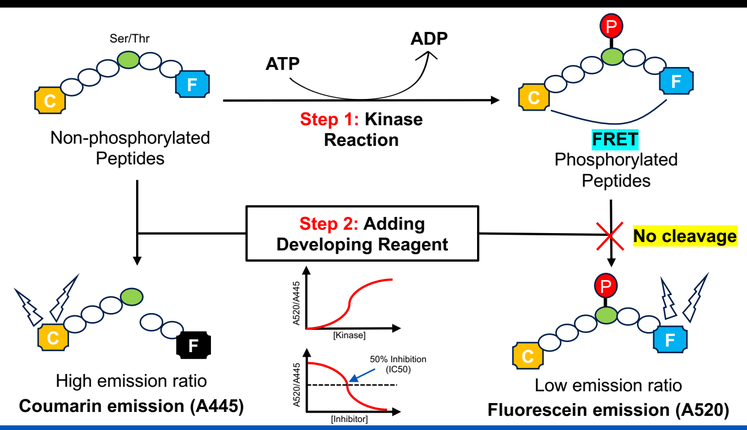




Lipid-activated regulation. Phospholipid membrane localization and Ca^{2+}-mediated interaction of PKCβ1 changes its conformation by opening the kinase domain and promoting ATP/substrate binding.

***Research question:** What are the structural features (shape, geometry, functional group, position) of Z-ENDX that help it bind and inhibit PKCβ1 & How does alteration of these features affect the inhibition?*

Z-LYTE Kinase Activity Assay



Selective Estrogen Receptor Modulators (SERMs)

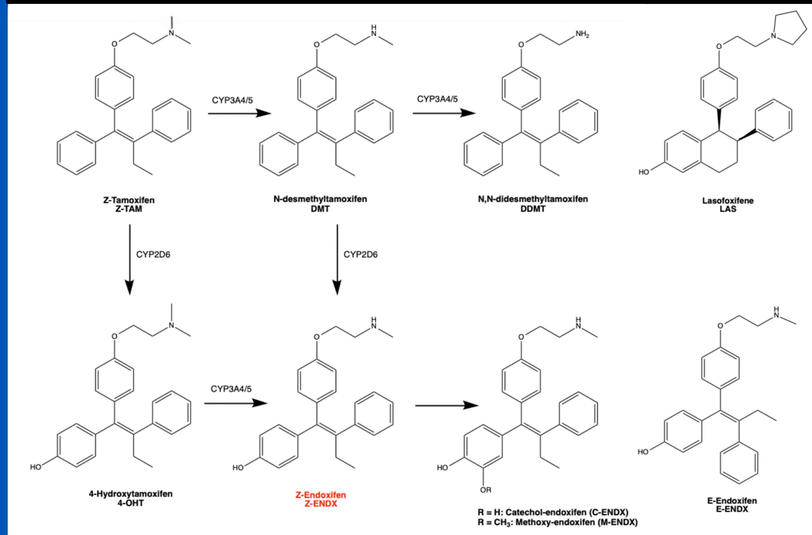


Figure 1. Z-TAM, Z-ENDX, and related metabolites as SERMs. Z-TAM can be metabolized into Z-ENDX through a set of Cytochromes: CYP3A4/5 (removing a N-methyl group) and CYP2D6 (adding a 4-hydroxy group), with DMT and 4-OHT as intermediates. Z-TAM, Z-ENDX and derivatives exhibit high antitumor activity in endocrine-resistant hormone receptor-positive breast (ER+ BC) and other gynecologic cancer. Our previous data also suggested Z-ENDX bind and inhibit PKCβ1, an oncogenic signaling kinase regulating cell proliferation as well as tumor transformation.[2] The molecules are drawn using ChemDraw 10.7.

Structure Activity Relationships

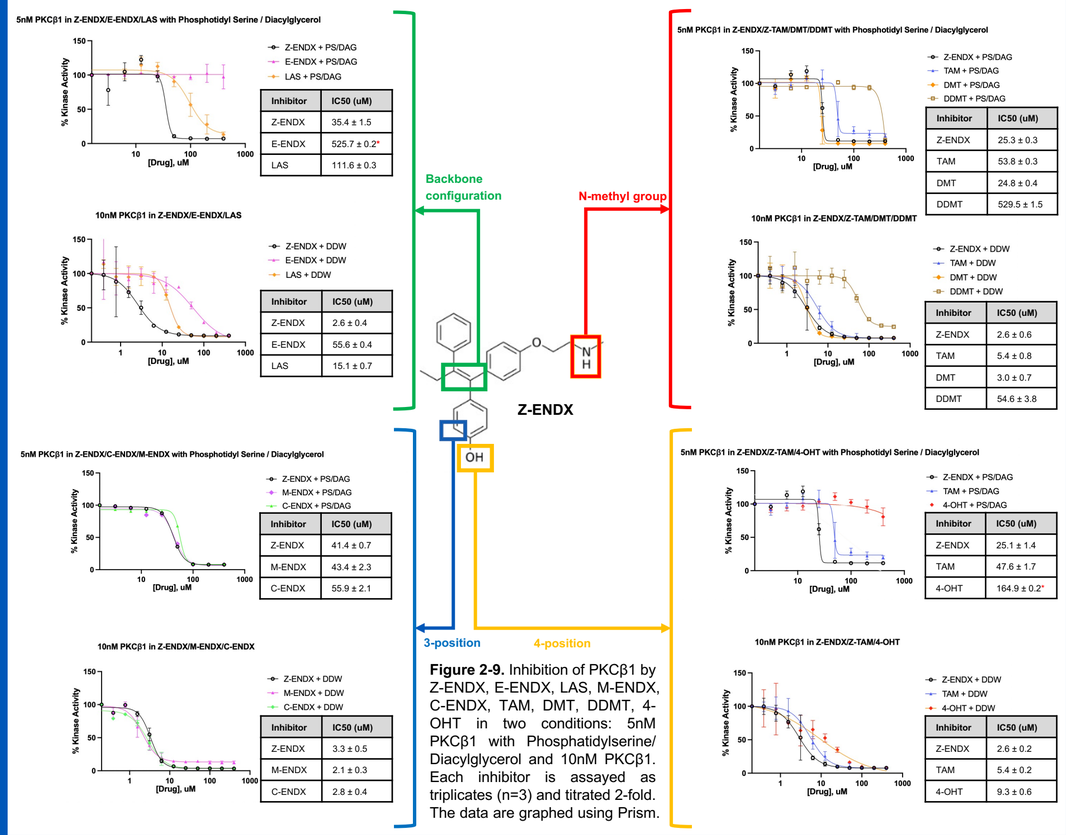


Figure 2-9. Inhibition of PKCβ1 by Z-ENDX, E-ENDX, LAS, M-ENDX, C-ENDX, TAM, DMT, DDMT, 4-OHT in two conditions: 5nM PKCβ1 with Phosphatidylserine/ Diacylglycerol and 10nM PKCβ1. Each inhibitor is assayed as triplicates (n=3) and titrated 2-fold. The data are graphed using Prism.

Conclusions

- In presence of Phosphatidylserine/Diacylglycerol, IC50 values of all inhibitors on PKCβ1 are higher than in the absence of lipids, suggesting that these inhibitors partially compete with lipid molecules in binding PKCβ1.
- 4-OHT loses potency compared to Z-ENDX and Z-TAM, while DMT maintains the same IC50 as Z-ENDX in both conditions, suggesting that removal of the N-methyl group is important for inhibitors binding to PKCβ1. On the other hand, the 4-OH group has been shown to be effective in binding estrogen receptors (ER).
- DDMT loses its potency and C-ENDX/M-ENDX shows the same potency as Z-ENDX, indicating that removing both N-methyl groups or adding other functional groups onto 3-position is disfavorable for PKCβI inhibition.
- Shapes and geometries are important, as both E-ENDX (E-isomer) and LAS (Tetrahedral) show less inhibition than Z-ENDX (Z-isomer, Trigonal planar).

Future Directions

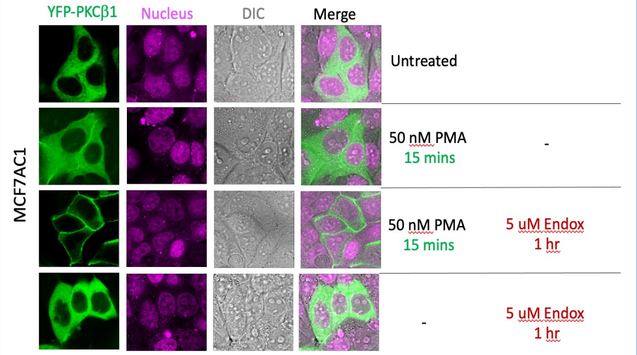


Figure 10. Z-ENDX triggers translocation PKCβ1 to the plasma membrane in presence of an PKC activator – Phorbol 12-myristate 13-acetate (PMA). In the absence of either Z-ENDX or PMA, there is not much change in PKCβ1 membrane localization. The endocrine-naïve breast cancer cell line MCF7AC1 was used in this experiment.

- Perform the same fluorescence microscopy experiment with the other inhibitors, including Z-TAM and its metabolites. This will potentially show the relationship between activity inhibition and plasma membrane localization of PKCβ1.
- Perform Z-LYTE kinase assay of PKCβ1 in presence of mixture of inhibitors (varying ratios), and compare their competition in binding PKCβ1. This can further confirm the potency of these inhibitors, as well as their binding specificities.
- Perform proliferation assay to see if in presence of different PKCβ1 inhibitors, cell growth could be affected.
- Perform X-ray crystallography on different SERMs inhibiting PKCβ1 to view the 3-D structure of their bindings.

Acknowledgements

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Reference:

1. Gundimeda, U., Chen, Z. H. & Gopalakrishna, R. Tamoxifen modulates protein kinase C via oxidative stress in estrogen receptor-negative breast cancer cells. J Biol Chem 271,13504-13514 (1996) doi.org:10.1074/jbc.271.23.13504.
2. Goetz, M. P. et al. First-in-Human Phase I Study of the Tamoxifen Metabolite Z-Endoxifen in Women With Endocrine-Refractory Metastatic Breast Cancer. J Clin Oncol 35, 3391-3400 (2017). https://doi.org:10.1200/jco.2017.73.3246