Molecular Pharmacology

The Structure-Function Relationship of Angular Estrogens and Estrogen Receptor Alpha to Initiate Estrogen-Induced Apoptosis in Breast Cancer Cells.

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Supplemental figures and tables

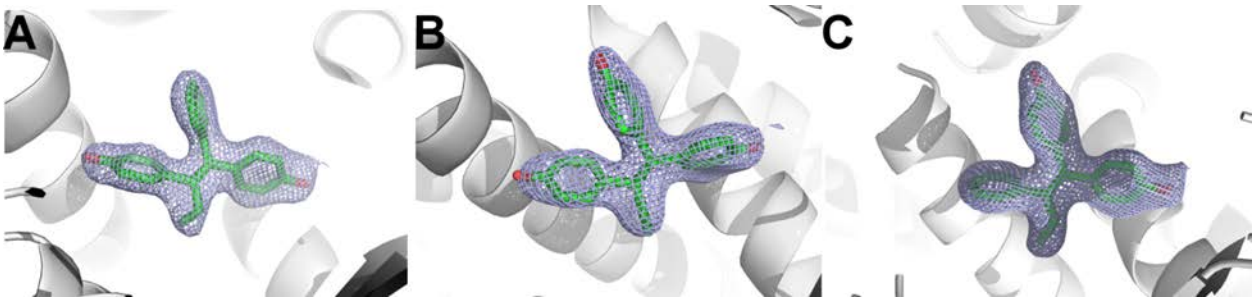

Fig. S1- 2mFo-DFc map showing the observed electron density for Z2OHTPE (A), 3OHTPE (B), and BPTPE (C) in complex with ER LBD contoured to 1.5σ. PBDs codes are 6CZN, 6D0F and 6D2A respectively.

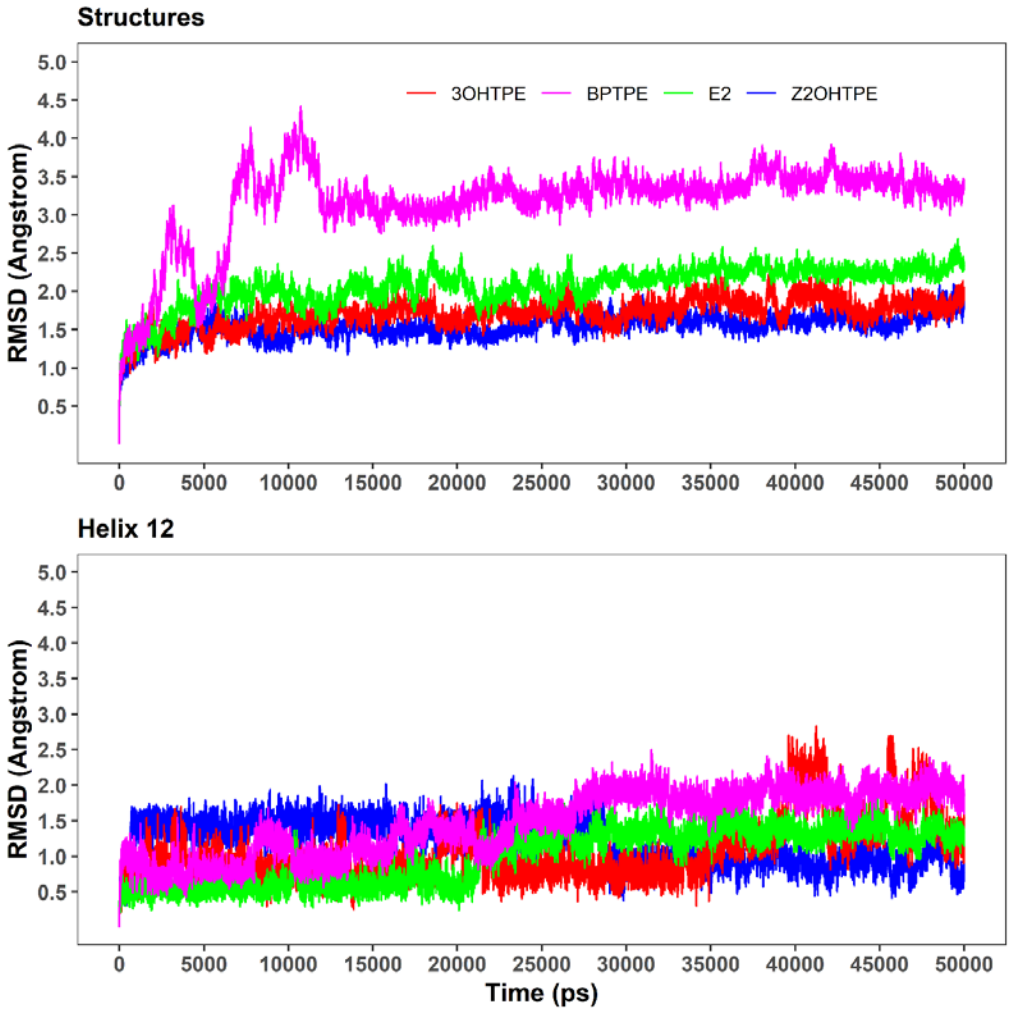

Figure S2 – Plots showing the RMSD evolution of the ER LBD WT in complex with E2 (green), Z2OHTPE (blue), 3OHTPE (red), and BPTPE (magenta), together with helix 12 for a simulation time of 50ns for each structure. RMSD is calculated based on the alignment of Cα atoms of each protein frame to the reference structure, first frame.

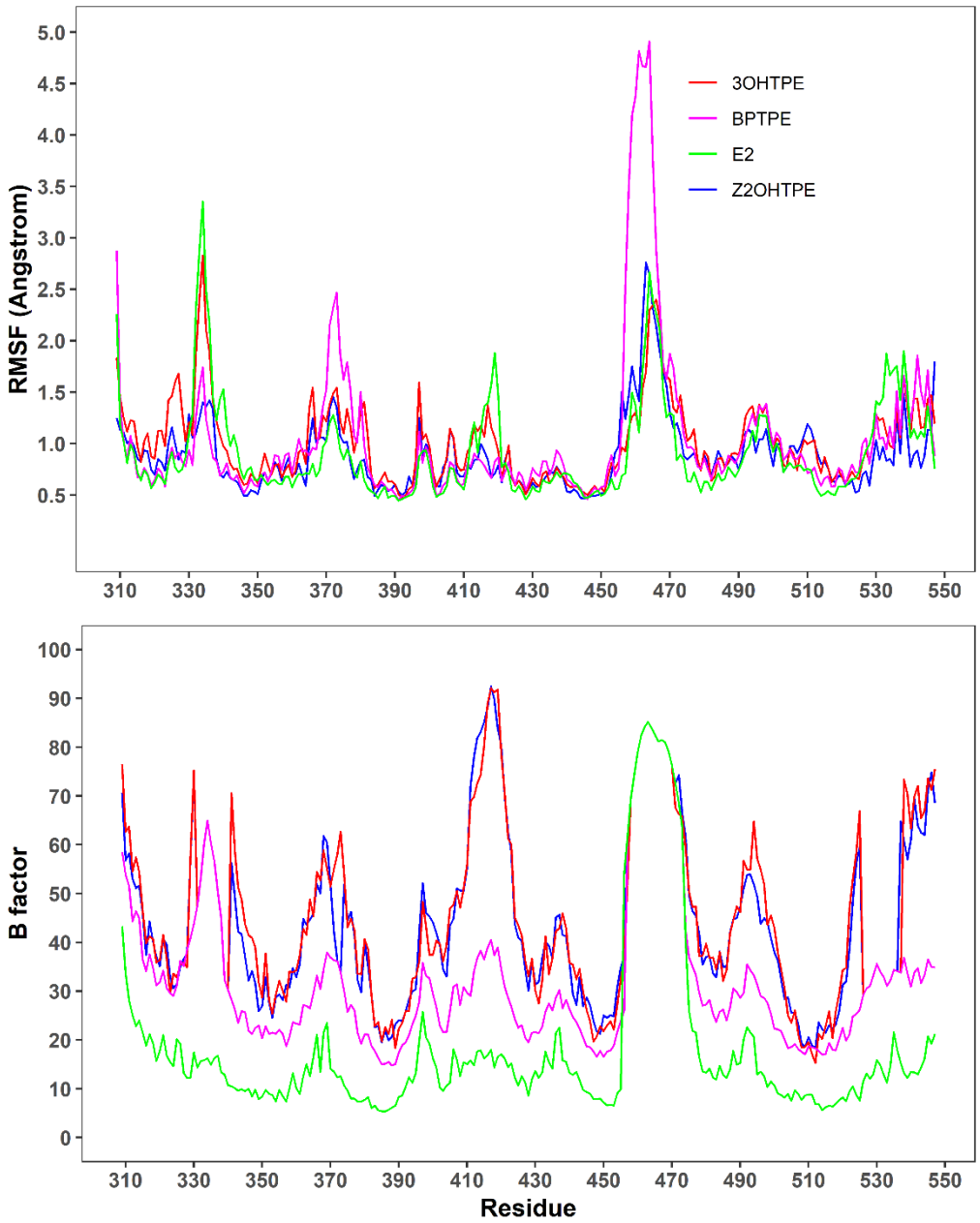

Fig. S3 – The root mean square fluctuation (RMSF in Å) of each residue for ER LBD WT bound complexes: E2 (green), Z2OHTPE (blue), 3OHTPE (red) and BPTPE (magenta), calculated for Cα atoms, during the 50ns simulation time. Also, the experimental B factors are shown to highlight the regions expected to fluctuate

more in the simulation.

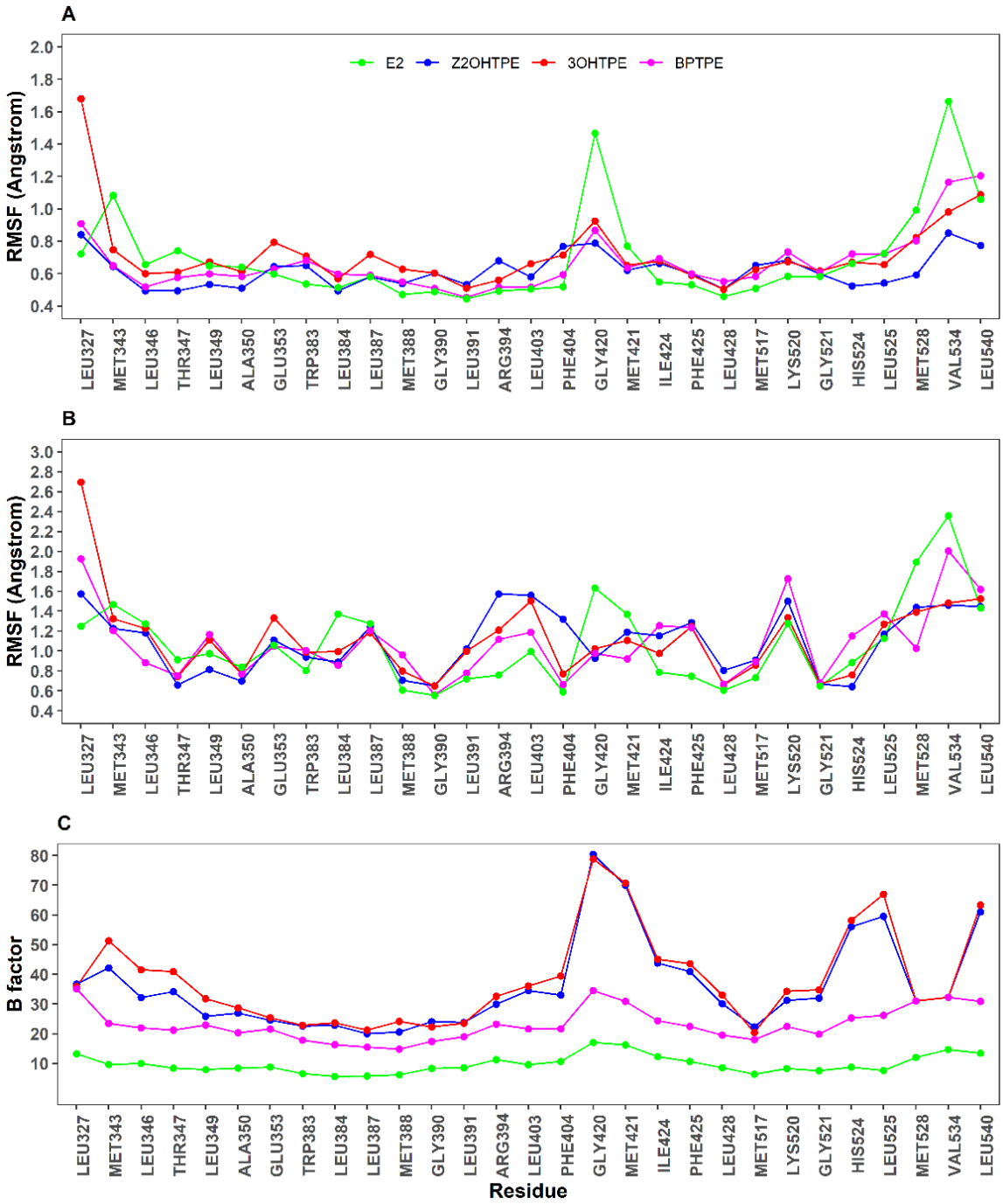

Figure S4 – RMSF calculated based on Calpha (A), side chains (B), and B factors (C) for amino acids lining the binding site.

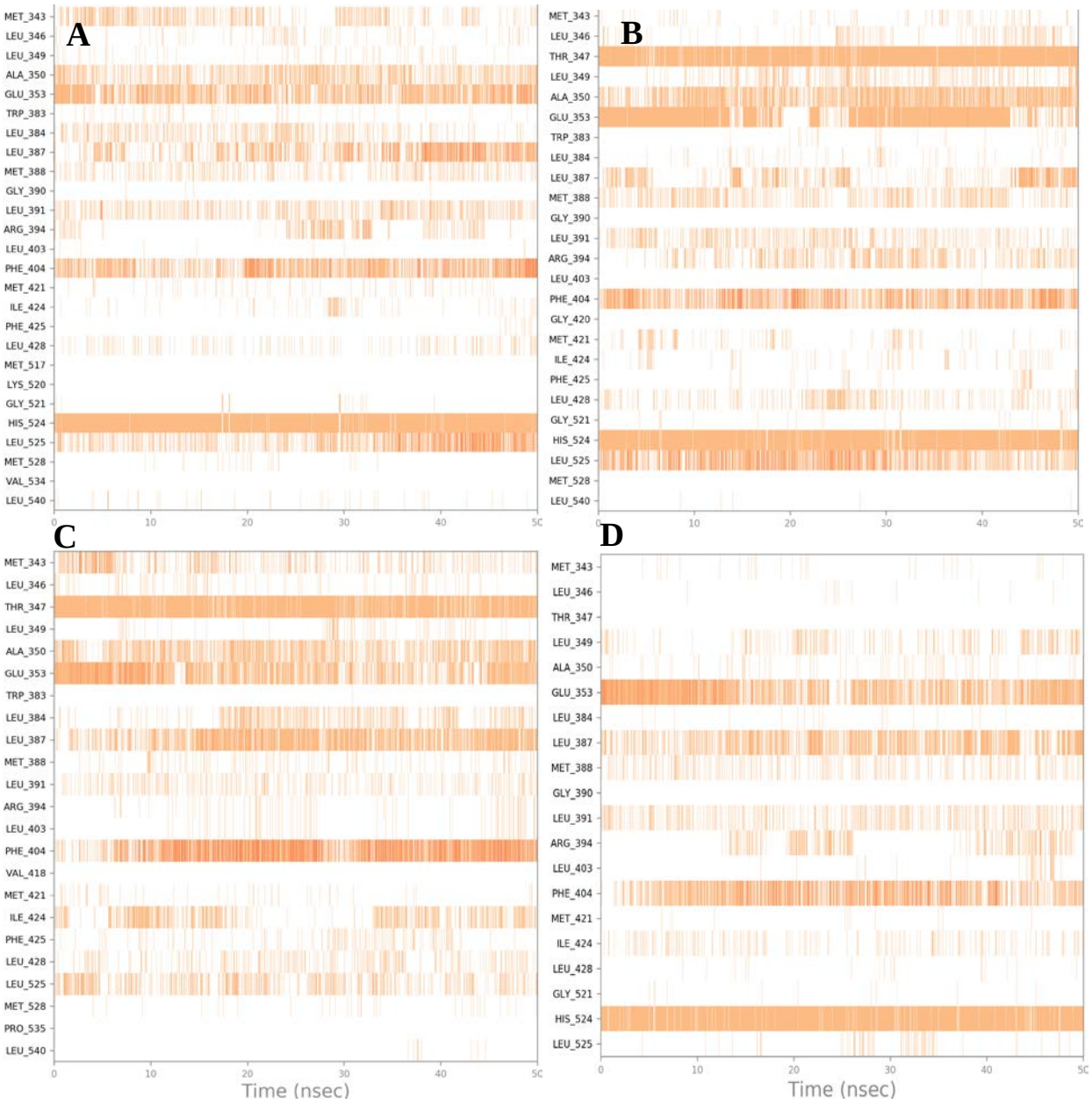

Figure S5 - A timeline representation of the interactions and contacts (H-bonds, hydrophobic, ionic, water bridges) in the binding sites. The panels show which residues interact with 2OHTPE (A), 3OHTPE (B), BPTPE (C), and E2 (D) in each trajectory frame, during the 50ns simulation time.

	ERα LBD Y537S-Z2OHTPE-GRIP	ERα LBD Y537S-3OHTPE-GRIP	ERα LBD Y537S-BPTPE-SRC2 SP4
PDB Code	6CZN	6D0F	6D2A
Data Collection			
Space Group	$P2_1$	$P2_1$	$P2_1$
a, b, c (Å)	53.84,81.66, 58.38	54.007, 82.992, 58.587	55.81, 82.583, 58.308
α, β, γ (°)	90, 111.07, 90	90, 111.04, 90	90, 109.63, 90
Resolution Range	46.10 - 2.5	43.08 - 2.5	
Number of Reflections			
(all/unique)	21750/6214	44652/14884	33735/9370
I/σI (highest resolution)	19.5 (1.89)	13.58 (2.82)	40.2 (2.39)
R_{merge} (highest resolution)	0.05 (0.47)	0.06 (0.36)	0.05 (0.6)
% Completeness (highest resolution)	88.8 (88.6)	82.4 (82.4)	99.6 (99.5)
Redundancy (highest resolution)	3.5 (2.8)	3.5 (2.5)	3.6 (3.6)
Refinement			
Rwork/Rfree (Highest Resolution)	19.6/23.8 (26.90/28.04)	21.0/24.1 (30.08/35.78)	21.35/26.28 (28.6/31.42)
No. Residues/Chain			
ERα LBD	423	445	429
Peptide	9	8	1
Protein β-factor	52.40	53.70	34.5
Water	64	63	105
Water β-factor	36.70	41.30	33.6
Ligand	2	1	1
Ligand β-factor	36.70	47.10	26.2
RMSD			
Bond lengths (Å)	0.03	0.003	0.01
Bond angles (°)	0.56	0.73	1.40
Ramachandran plot statistics			
Preferred number (%)	415 (98.11 %)	417 (99.05%)	401 (99.01%)
Additional allowed (%)	8 (1.89%)	4 (0.95%)	4 (0.99%)
Outliers (%)	0	0	0

Table S1. Data collection and refinement statistics for the X-ray crystallographic structures.

	RMSD (Å)			
ERα-	Cα atoms protein	Cα atoms H12	Lig fit Lig*	Lig fit Prot**
E2	2.13±0.17	1.06±0.37	0.23±0.06	1.04±0.29
Z2OHTPE	1.57±0.13	0.93±0.15	0.41±0.09	1.43±0.21
3OHTPE	1.75±0.15	1.36±0.49	0.47±0.17	1.11±0.26
BPTPE	3.14±0.14	1.89	0.4±0.13	1.1±0.24

*RMSD of a ligand that is aligned and measured just on its reference conformation, it measures the internal fluctuations of the ligand atoms.

**RMSD of a ligand when the protein-ligand complex is first aligned on the protein backbone of the reference, and then the RMSD of the ligand heavy atoms is measured. If the RMSD values observed are significantly larger than the RMSD of the protein, the ligand has likely diffused away from the initial binding site.

Table S2. Average RMSD for several parameters calculated over the 50ns trajectories.

ERα-	Thr347-lig (OG1…H-O)	Glu353-lig (Oε1/ε2…H-O)	Arg394-H_2O-lig (NH2+…O…H-O)	His524-lig (Nδ/ε…H-O)	Phe404
E2	0%	94%	15%	96%	56%
Z2OHTPE	0%	75%	12%	98%	20%
3OHTPE	94%	66%	22%	96%	24%
BPTPE	90%	65%	5%	0%	47%

Table S3. Occurrence of ligand-residues interactions (%) during the 50ns simulation time.

Supplemental File 1. The list of all genes expression levels included in the microarray heatmap in figure 5.

Supplemental File 2. List of all peptides identified by mass spectrometry and their data for figure 8.