

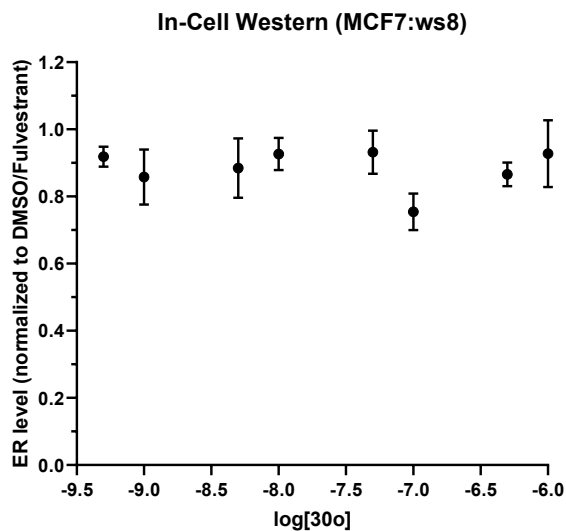
Supporting Information

Design and Synthesis of Basic Selective Estrogen Receptor Degraders (B-SERDs) for Endocrine Therapy Resistant Breast Cancer

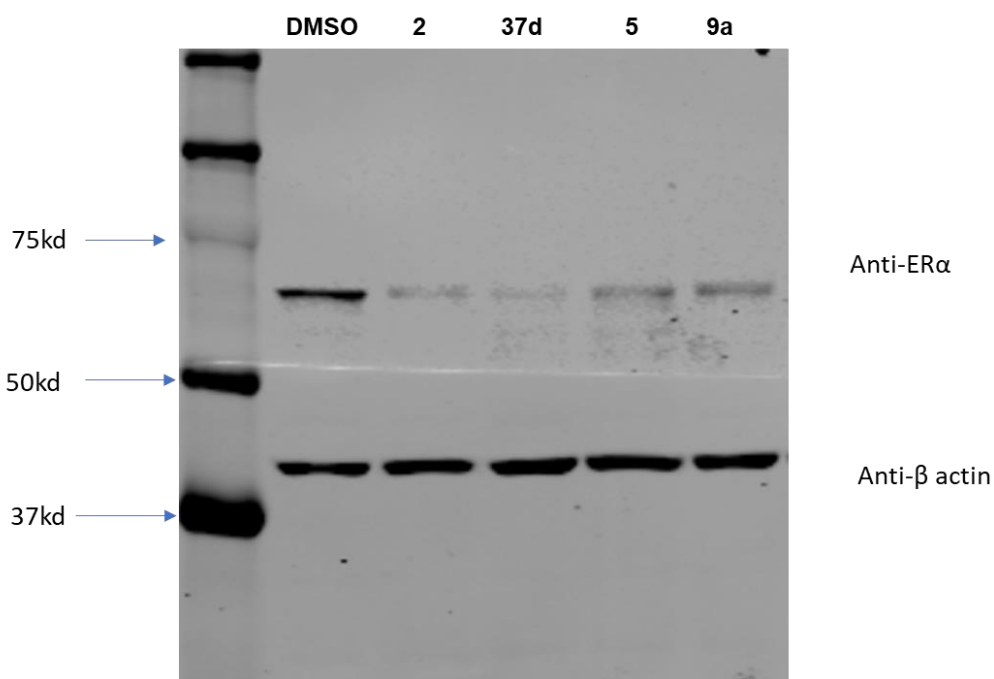
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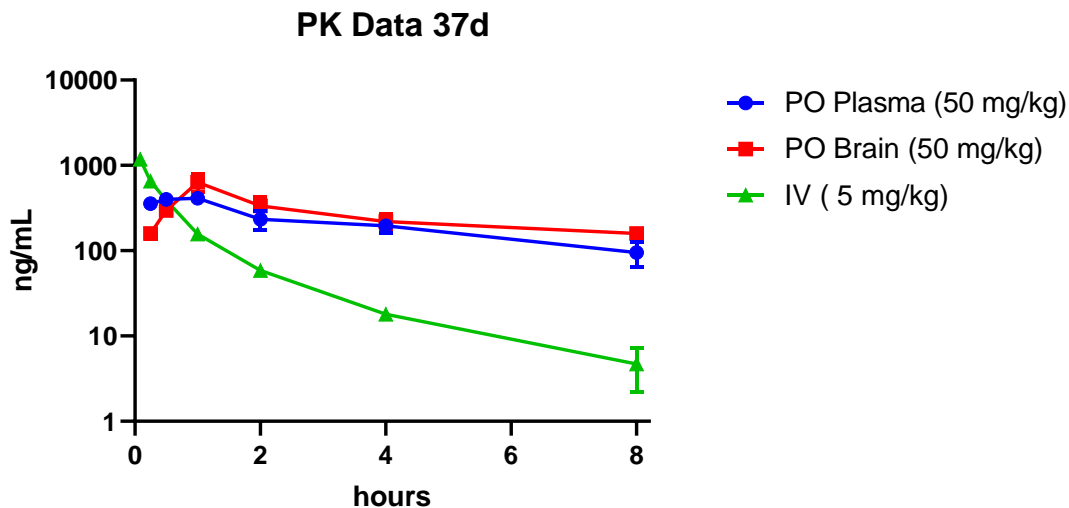
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Supplementary Figure 1. Effect of 30o treatment on ER Level. ER α level in MCF7:WS8 cells treated with **30o** for 24 hours. Normalized to vehicle (1) and 1 μ M **2** (0). Data shown as mean \pm SEM from analytical triplicate.



Supplementary Figure 2. Representative Full Western Blots. MCF-7:WS8 cells were treated with test compounds (100nM) for 24h. Primary Ab (Cell Signaling): anti-ER α and β -actin; Secondary Ab: anti-rabbit and mouse.



Supplementary Figure 3. PK profiles for 37d. Data show mean and S.E.M. from LC-MS/MS measurements.

Supplementary Table 1. ER isoform binding specificity

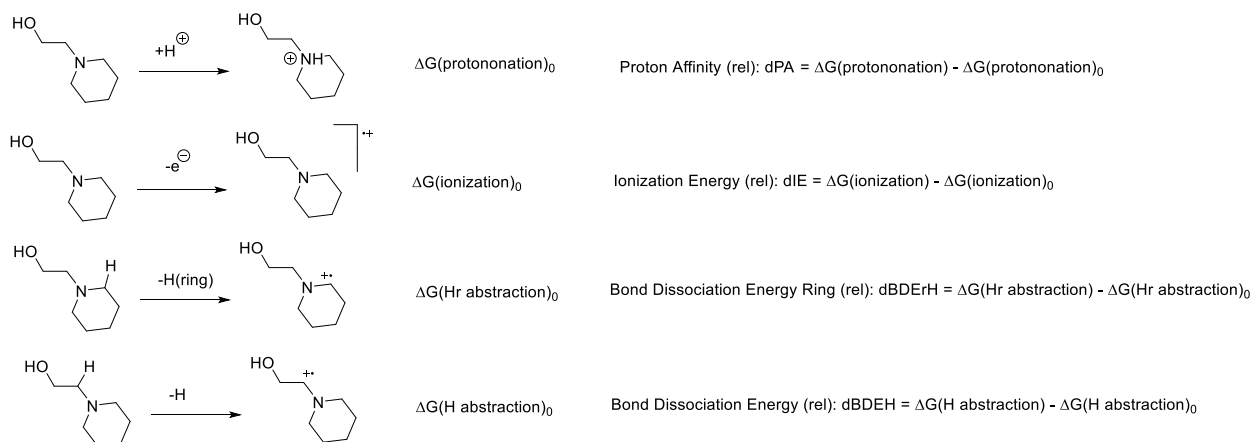
Compound	RBA ER α (%)	RBA ER β (%)	α/β
30c	80.56 \pm 20	52.19 \pm 0.4	1.5
30d	30.70 \pm 7	16.73 \pm 2	1.8
30e	46.49 \pm 7	22.29 \pm 0.3	2.1
30f	59.58 \pm 3	13.52 \pm 3	4.4
30g	56.96 \pm 14	19.21 \pm 3	3.0
30h	21.93 \pm 3	11.21 \pm 3	2.0
30i	9.15 \pm 0.87	8.87 \pm 3	1.0
30j	119.86 \pm 13	32.19 \pm 5	3.7
30m	32.64 \pm 6	16.40 \pm 4	2.0
37b	91.79 \pm 6	34.32 \pm 6	2.7
37d	27.78 \pm 8	17.19 \pm 2	1.6
37f	38.09 \pm 9	28.67 \pm 5	1.3

Relative binding affinity (RBA) values, determined by radioligand displacement assays expressed as $IC_{50} \text{ estradiol} / IC_{50} \text{ compound} \times 100$ (RBA, estradiol = 100%).

DFT molecular orbital calculations

To model the electronic properties of the putative B-SERD side arms, simplified structures were used as shown in Figure 2. The conformational space for each neutral, ground state structure was explored and structures representing local energy minima stabilized by intramolecular interactions were excluded to better mimic the extended conformations anticipated on the ligand binding pocket. In several cases, 2 or 3 conformations representing local minima were identified. Conformational analysis was performed at the B3LYP/6-31+G** level in Spartan 10 from Wavefunction Inc. Structures for protonated and ionized amine side arms were also optimized at the B3LYP/6-31+G** level. Full thermodynamic calculations (298.15° C), corrected for ZPE were performed at the RI-MP2/6-311++G** level on structures optimized at the lower level. The composite RI-MP2/6-311++G**//B3LYP/6-31+G** level of calculation is suitable for energy calculations on radical and charged species.

The following reactions (shown for the piperidine side arm only) were used to calculate free energy changes:



ΔG for the piperidine reactions was set as $\Delta G_0 = 0$. The dG was then calculated using calculated ΔG for each reaction and side arm: as shown above for dPA, dIE, dBDErH (ring H abstraction), and dBDEH. Where multiple conformers were identified, the free energy difference is given in Figure 2 for the lowest energy reaction.

