Boron-based 4-Hydroxtamoxifen Bioisosteres for Treatment of de novo Tamoxifen Resistant Breast Cancer

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Supporting Information

EXPERIMENTAL PROCEDURE

All reagents and solvents were purchased from Sigma-Aldrich Chemical Co., Fisher Scientific, AK Scientific, and CombiPhos Catalyst and were used as received. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AX 400 spectrometer or Varian Inova-300 spectrometer. Chemical shift are reported as parts per million (ppm) relative to TMS. Mass spectral data were collected on a Shimadzu LC-MS 2010 instrument and HRMS spectra data were collected on a Thermo LTQ Orbitrap-XL mass spectrometer in positive or negative ion modes. Unless specified otherwise, all tested compounds were confirmed to be >95% pure by HPLC.

MCF-7 cell line was purchased from ATCC (ATCC #HTB-22, Manassas, VA). T47D cell line was a gift from Dr. Thomas Wiese of Xavier University of Louisiana. MDA-MB-231 cell line was a gift from Dr. KiTani Johnson of Xavier University of Louisiana. These breast cancer cell lines were routinely cultured in phenol red-free DMEM medium supplemented with 5% FBS, 4mM glutamine, 1mM sodium pyruvate, 100IU/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin. Cultures were maintained in 5% carbon dioxide at a temperature of 37°C.

For growth assay in the presence of tamoxifen, 4-OHT or prodrugs, MCF-7 and T47D cells were plated in six-well plates at a density of 50,000 each well in 5% FBS DMEM medium. The cells were then treated with tamoxifen, 4-OHT, or prodrugs separately at 6 different doses ranging from 10^{-8} M to 5×10^{-6} M for 5 days, while equal treatment volumes of DMSO were used as vehicle control. Cell numbers were counted with a Coulter instrument (Beckman-Coulter). The ratio of drug treated cell numbers to vehicle treated cell numbers was defined as survival ratio. IC 50 values were obtained from dose-response curves for each boron prodrug. Experiments were conducted in triplicate and data represented as mean ± SD. For measurement of drug uptake by breast cancer cells, MCF-7 cells were incubated with 4-OHT or a boron

prodrug at 10^{-6} M. After 6 days, the cell culture media were collected, centrifuged to remove cells, and diluted to a fixed volume for relative quantitation by HPLC-MS/MS. Chromatographic peak areas corresponding to the prodrug and 4-OHT were used as concentrations in the media. For measurement of the half-lives of the boron prodrugs, each drug was incubated with MCF-7 cells in 12- well plates at a concentration of 10^{-6} M. Media samples were collected at 11 time points from 0, 1 , 2, 4, 8, 16, 24, 28, 72, 96, 144 hrs. Media samples were immediately mixed with 1 mL of chilled methanol, centrifuged, and brought to a final volume of 3 mL for each sample before HPLC-MS/MS analysis.

Synthesis of 4, 4'-(2-phenylbut-1-ene-1,1-diyl)diphenol (1): To a suspension of zinc (10.0 g, 0.16 mol) in dry THF (80 mL) was added dropwise $TiCl_4$ (7.5 mL, 0.07 mol) under nitrogen at -10 ^oC. When the addition was complete, the mixture was warmed to room temperature and then refluxed for 2h. To the cooled suspension of the titanium reagent was added a solution of 4, 4'-hydroxybenzophenone (2.5 g, 0.012 mol) and propiophenone (5.0 g, 0.037 mol) in dry THF (50 mL) at 0 ^oC, and the mixture was refluxed in the dark for 2h. After being cooled to rt, the reaction mixture was quenched with brine (10 mL) and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Flash column chromatography (Hexane: EtOAc, 8:2) afforded **1** (3.55g) in 96 % yield as a white solid. ^{1}H-NMR ($CDCl_3$): 7.16-7.09 (7H, m), 6.80 (2H, d, J = 8.0 Hz), 6.72 (2H, d, J = 8.4 Hz), 6.47 (2H, d, J = 8.4 Hz), 4.84 (1H, s), 4.60 (1H, s), 2.48 (2H, q, J = 7.2 Hz), 0.92 (3H, t, J = 7.2 Hz). ESI (-): 315.17 (M-H).

Synthesis of (*E,Z*)-4-Hydroxytamoxifen (2): A solution of 4,4'-(2-phenylbut-1-ene-1,1-diyl)diphenol (**1**) (0.90 g, 0.0028mol) in DMF (10 mL) was treated with Cs_2CO_3 (2.12 g, 0.0066mol) and heated in an oil bath at 70-80 ^oC for 10 min. The resulting suspension was treated with 2-(dimethylamino) ethyl chloride hydrochloride (1.5 g, 0.01mol) in a small portion

over a 15 min period and stirred for 1.5 h. After being cooled to rt, the reaction mixture was poured into saturated ammonium chloride (10 mL) and extracted with ethyl acetate (4 × 10 mL). The combined organic phase was washed with brine (4 × 10 mL), dried, and concentrated in vacuo. Flash column chromatography (CH_2Cl_2: MeOH, 9:1) afforded **2** (0.30 g) in 30 % yield as a 1:1 mixture of (E)/(Z)-isomers. ^{1}H-NMR (CD_3OD): 7.15-7.07 (6H, m), 7.01 (1H, d, *J* = 8.4 Hz), 6.93 (1H, d, *J* = 8.8 Hz), 6.77-6.74 (2H, m), 6.64 (1H, d, *J* = 8.8 Hz), 6.56 (1H, d, *J* = 8.8 Hz), 6.39 (1H, d, *J* = 8.8 Hz), 4.59 (1H, s, br), 4.12 (1H, t, *J* = 5.6 Hz), 3.96 (1H, t, *J* = 5.6 Hz), 2.80 (1H, t, *J* = 5.6 Hz), 2.69 (1H, t, *J* = 5.6 Hz), 2.49-2.45 (2H, m), 2.36 (3H, s), 2.30 (3H, s), 0.90 (3H, t, *J* = 7.2 Hz). ESI (+): 388.25 (M+H).

Synthesis of (*E*,*Z*)-4-(1-(4-(2-(dimethylamino)ethoxy)phenyl)-2-phenylbut-1-en-1-yl)phenyl trifluoromethanesulfonate (3): A solution of trifluoromethanesulfonic anhydride (0.28g, 0.17 mL, 1.0 mmol) in CH_2Cl_2 (5.0 mL) was added dropwise to a solution of pyridine (0.1 mL, 1.2 mmol) and 4-hydroxytamoxifen (**2**) (0.30g, 0.77 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. After complete addition, the mixture was warmed to room temperature and allowed to stir for 1 hour. The mixture was then diluted with Et_2O, quenched with 10 % aq HCl and washed successively with sat. $NaHCO_3$ and brine. After drying over $MgSO_4$, the solvent was removed under reduced pressure and the residue was purified by flash column chromatography to give the triflates **3** (0.38g) in 95% yield. ^{1}H-NMR ($CDCl_3$): 7.32 (1H, d, *J* = 8.8 Hz), 7.25 (1H, d, *J* = 8.8 Hz), 7.18-7.08 (5H, m), 7.05 (1H, d, *J* = 8.8 Hz), 6.93-6.88 (3H, m), 6.75 (1H, d, *J* = 8.8 Hz), 6.56 (1H, d, *J* = 8.8 Hz), 4.19 (1H, t, *J* = 5.6 Hz), 4.03 (1H, t, *J* = 5.6 Hz), 2.98 (1H, t, *J* = 5.6 Hz), 2.86 (1H, t, *J* = 5.6 Hz), 2.53 (3H, s), 2.50-2.42 (2H, m), 2.47 (3H, s), 0.92 (3H, m).^{13}C-NMR ($CDCl_3$): 157.6, 156.7, 148.4, 147.6, 144.3, 144.1, 143.9, 143.1, 141.9, 141.8, 136.7, 136.5, 135.7, 135.4, 132.6, 132.1, 131.5, 130.9, 129.8, 129.7, 128.24,

128.22, 126.8, 126.6, 121.2, 120.3, 114.6, 113.8, 65.2, 64.9, 58.1, 58.0, 45.6, 45.5, 29.9, 29.3, 29.2, 13.71, 13.67. ESI (+): 520.17 (M+H).

Synthesis of *N*,*N*-dimethyl-2-(4-(2-phenyl-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)but-1-en-1-yl)phenoxy)ethanamine (4): To a 1,4-dioxane solution of the triflate **3** (0.30g, 0.57mmol) were added bis(pinacolato)diboron (0.16g, 0.63mmol), $PdCl_2$(dppf) (0.025g, 5% mol) and KOAc (0.14g, 1.43mmol), and the mixture was stirred under N_2 at 80 ^oC overnight. After the solution was cooled, the dioxane was removed under vacuum, and CH_2Cl_2 and water were added. The resulting mixture was extracted with dichloromethane twice, and the combined organic layer was washed with brine and then dried over MgSO4. The organic solvent was concentrated in vacuo. The crude was purified by flash chromatography to afford **4** (0.26g) in 90 % yield. ^{1}H-NMR ($CDCl_3$): 7.78 (1H, d, *J* = 8.0 Hz), 7.43 (1H, d, *J* = 7.6 Hz), 7.30-7.03 (7H, m), 6.91-6.84 (2H, m), 6.76 (1H, d, *J* = 8.4 Hz), 6.51 (1H, d, *J* = 8.4 Hz), 4.48 (1H, t, *J* = 5.6 Hz), 4.30 (1H, t, *J* = 5.6 Hz), 3.49 (1H, t, *J* = 5.6 Hz), 3.38 (1H, t, *J* = 5.6 Hz), 2.93 (3H, s), 2.85 (3H, s), 2.49-2.39 (2H, m), 1.33 (6H, s), 1.26 (3H, s), 0.92 (3H, m).^{13}C-NMR ($CDCl_3$): 156.1, 155.3, 146.8, 146.4, 143.1, 142.4, 142.3, 142.2, 138.1, 138.0, 137.5, 136.9, 134.9, 134.1, 132.6, 132.3, 131.11, 131.08, 130.4, 129.8, 129.7, 129.1, 128.3, 128.2, 128.1, 126.5, 120.4, 114.7, 114.4, 113.6, 84.0, 83.8, 63.2, 63.0, 62.7, 56.9, 56.8, 56.7, 44.1, 44.0, 43.9, 29.3, 25.11, 25.06, 13.8, 13.71, 13.65. ESI (+): 498.25 (M+H). HRMS (ESI(+)): Calcd. for $C_{32}H_{41}BNO_3$ (M+H) : 498.3179. Found: 498.3172.

Synthesis of Potassium (4-(1-(4-(2-(dimethylamino)ethoxy)phenyl)-2-phenylbut-1-en-1-yl)phenyl) trifluoroborate (5):

To a solution of the pinacolylboronate (0.26g, 0.52 mmol) in methanol was added aqueous potassium bifluoride (0.8 ml, 3M, 2.4 mmol). The resulting mixture was stirred at room

temperature for 15 min, concentrated in vacuo and dissolved in hot acetone. The mixture was filtered, the filtrate was concentrated in vacuo and the residue recrystallized from acetone and ether to afford potassium trifluoroborate **5** (0.16g) in 65 % yield as a crystalline solid. ^{1}H-NMR (CD_3CN): 7.46 (2H, d, *J* = 7.2 Hz), 7.23-7.11 (5H, m), 7.06 (2H, d, *J* = 7.6 Hz), 6.93 and 6.85(2H, d, *J* = 8.8 Hz), 6.72 and 6.60 (2H, d, *J* = 8.8 Hz), 4.17 and 4.02 (2H, t, *J* = 5.2 Hz), 3.16 and 3.08 (2H, t, *J* = 5.2 Hz), 2.65 and 2.59 (6H, s), 2.44 (2H, q, *J* = 7.2 Hz), 0.91 (3H, m). ^{13}C-NMR (CD_3CN): 156.9, 156.1, 143.3, 140.9, 139.6, 137.6, 137.3, 131.9, 131.5, 130.8, 130.6, 130.0, 129.9, 126.2, 115.6, 114.4, 113.6, 63.3, 57.3, 44.2, 29.0, 13.1.ESI (-): 438.25 (M-K). HRMS (ESI(+)): Calcd. for $C_{26}H_{29}BF_3KNO$ (M+H) : 478.1931. Found: 478.1944.

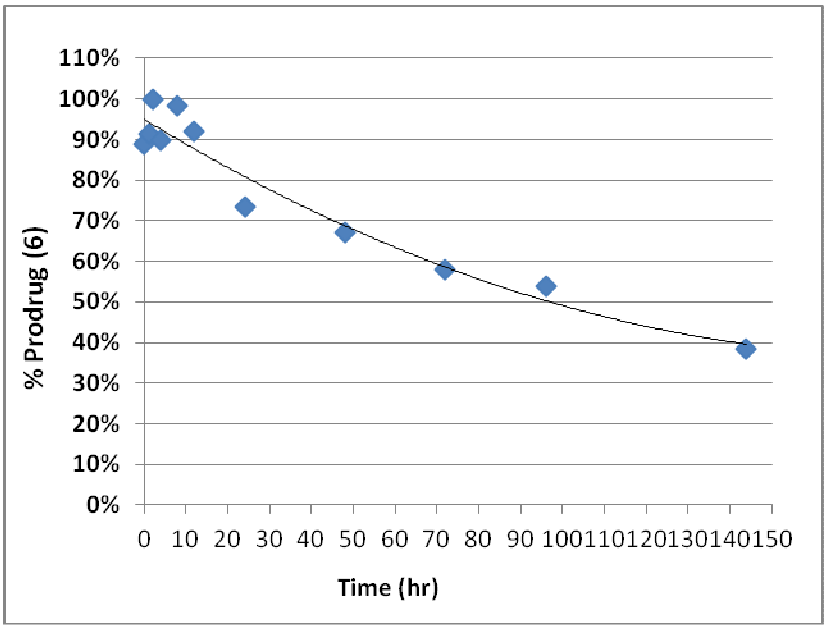
Synthesis of (4-(1-(4-(2-(dimethylamino)ethoxy)phenyl)-2-phenylbut-1-en-1-yl)phenyl) boronic acid (6):

To a solution of the potassium trifluoroborate **5** (0.16g, 0.33 mmol) in water and acetonitrile was added trimethylsilyl chloride (0.10g, 1.0 mmol). The resulting solution was stirred at room temperature for 1h, quenched with saturated sodium bicarbonate, and extracted with dichloromethane. The combined organic solution was dried over $MgSO_4$, filtered and concentrated in vacuo to afford the corresponding boronic acid **6** (0.13g) in 98% yield as a white solid. ^{1}H-NMR ($(CD_3)_2CO$): 7.90 (1H, d, *J* = 8.0 Hz), 7.54 (1H, d, *J* = 8.0 Hz), 7.27-7.02 (10H, m), 6.89 (1H, d, *J* = 8.0 Hz), 6.84 (1H, d, *J* = 8.8 Hz), 6.67 (1H, d, *J* = 8.8 Hz), 4.52 and 4.34 (2H, t, *J* = 4.8 Hz), 3.84 and 3.74 (2H, t, *J* = 4.8 Hz), 3.20 and 3.13 (6H, s), 2.46 (2H, m), 0.90 (3H, m). ^{13}C-NMR ($(CD_3)_2CO$): 156.0, 145.8, 142.5, 142.4, 141.8, 138.8, 138.7, 136.7, 135.5, 134.4, 133.6, 132.0, 130.7, 130.0, 129.9, 128.6, 128.2, 128.0, 126.4, 114.7, 113.8, 75.8,

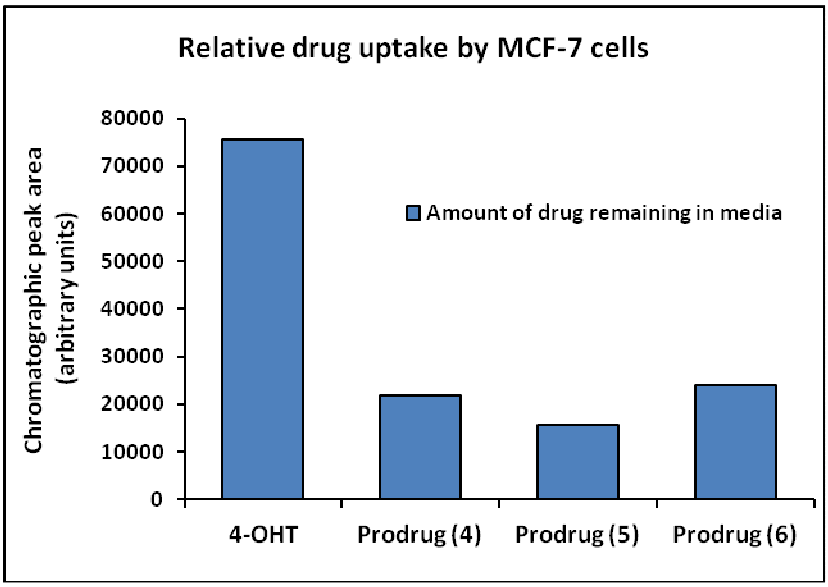
62.2, 62.0, 56.9, 43.6, 13.1. ESI (+): 416.25 (M+H). HRMS (ESI(+)): Calcd. for $C_{26}H_{31}BNO_3$ (M+H) : 416.2397. Found: 416.2392.

Reaction products were analyzed on the Shimadzu LC-MS system using a C18 column (Phenomenex, Torrance, CA). Cell culture media samples were analyzed on a linear ion trap tandem mass spectrometer (LTQ) interfaced with a Surveyor HPLC system (Thermo-Fisher Scientific, West Palm Beach, FL). Chromatographic separations were achieved by using a Synergi 4u Hydro-RP 80A column (150 X 2.0 mm, 4 µm particle size; Phenomenex, Torrance, CA). Samples were eluted from the analytical column with mobile phase A (water with 0.1% formic acid) and mobile phase B (acetonitrile with 0.1% formic acid) at a flow-rate of 0.2 mL/min over 22 min using a linear gradient of 0–100% B for 13 min; holding at 100% B for 5 min before returning to 0% B.

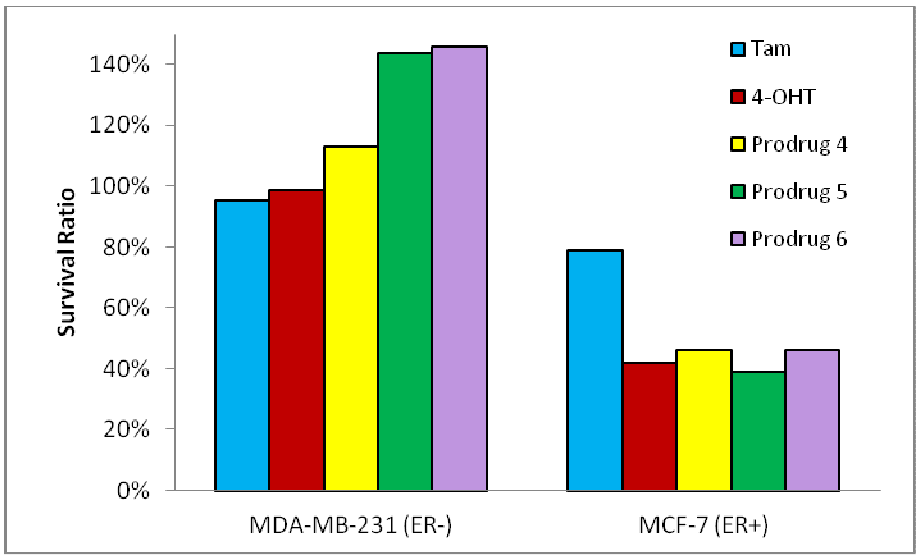
For analysis of the relative concentrations of prodrugs and 4-hydroxytamoxifen, selected ion monitoring (SIM) method was used where m/z 416 was monitored for the boronic acid prodrug (**6**), m/z 440 for prodrug (5), m/z498 for prodrug (4), and m/z388 for 4-hydroxytamoxifen. The peak areas of the above mentioned ions were recorded and used to calculate the relative concentrations of their corresponding molecules.



Supplemental Figure 1S. Time dependent conversion of prodrug (6) to 4-OHT in MCF-7 cell culture.



Supplemental Figure 2S. Prodrug uptake by MCF-7 cells as measured by total (4-OHT plus prodrug) remaining concentration in media.



Supplemental Figure 3S. Effect of prodrugs on cell growth in ER negative (MDA-MB-231) and ER positive (MCF-7) breast cancer cells.