Supporting Information for

6-Aryl-1,4-dihydro-benzo[*d*][1,3]oxazin-2-ones. A Novel Class of Potent, Selective, and Orally Active Nonsteroidal Progesterone Receptor Antagonists

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6-Bromo-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]oxazin-2-one (5). To a solution of 2-(2-amino-5-bromophenyl)propan-2-ol (18g, 78 mmol) in dry THF (150 mL) was added 1,1'-carbonyldiimidazole (15.5g, 94 mmol) under nitrogen. The reaction solution was heated at 50 °C overnight. The solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate (100 mL). The solution was washed with 1N aqueous hydrochloride solution (2 x 40 mL), brine (20 mL), and dried with $MgSO_4$. After removal of solvent *in vacuo*, 6-bromo-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]oxazin-2-one was obtained as a white solid (20 g, 100%): mp 199-200 °C; ^{1}H-NMR (DMSO-d_6) 10.32 (s, 1H, D_2O exchangeable), 7.48 (d, 1H, J = 2.1 Hz), 7.43 (dd, 1H, J = 8.5, 2.1 Hz), 6.84 (d, 1H, J = 8.4 Hz), 1.61 (s, 6H). Anal. calcd for $C_{10}H_{10}BrNO_2$: C:46.90 H:3.94 N:5.47 Found: C:46.83 H:3.83 N:5.45.

(1,4-Dihydro-4,4-dimethyl-2-oxo-2H-3,1-benzoxazin-6-yl)boronic acid (6). To a solution of 6-bromo-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]oxazin-2-one (2g, 7.8 mmol) in anhydrous THF (60 mL) was added a solution of *n*-BuLi in hexane (10 M, 2.4 mL, 24 mmol) at -78 °C under nitrogen. After stirring at -78 °C for 30 minutes, The solution was treated with triisopropyl borate (6.5 mL, 28 mmol). The reaction medium was slowly warmed to ambient temperature and quenched with 1N aqueous hydrochloric acid solution (60 mL). Ethyl acetate (100 mL) was added and organic layer was separated, and aqueous layer was extracted with ethyl acetate (3 x 60 mL). The combined organic layer was washed with brine and dried with $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by a silica gel flash chromatography (ethyl acetate:hexane/2:1) to afford (1,4-dihydro-4,4-dimethyl-2-oxo-2H-3,1-benzoxazin-6-yl)boronic acid as a white solid (1.4g, 81%): mp 249-250 °C; ^{1}H-NMR (DMSO-d_6) 10.21 (s, 1H, D_2O exchangeable), 7.90-7.95 (br s, 2H, D_2O exchangeable), 7.67 (m, 2H), 6.79 (d, 1H, J = 7.8 Hz), 1.61 (s, 6H); MS (ESI) *m/z* 222 ($[M+H]^+$, 87%).

6-(3-Chlorophenyl)-4,4-dimethyl-1,4-dihydrobenzo[d][1,3]oxazin-2-one (4c, Procedure A). A mixture of 6-bromo-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]oxazin-2-one (1.5g, 5.9

mmol), 3-chlorophenyl boronic acid (1.83g, 11.7 mmol), tetrakis(triphenylphosphine)-palladium (0) (0.35g, 0.3 mmol), and sodium carbonate (2.48g, 23.4 mmol) in a mixture of DME and water (40 mL/10 mL) was degassed to remove the oxygen and then heated at 85 °C under a blanket of nitrogen for 3 hours. The reaction mixture was cooled to ambient temperature and quenched with a saturated aqueous ammonium chloride solution (20 mL). Ethyl acetate (50 mL) was added and organic layer was separated. The aqueous layer was extracted with ethyl acetate (3x15 mL). The combined organic layers were washed with brine and dried with $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by a silica gel flash chromatography (hexane:ethyl acetate/2:1) to afford 6-(3-chlorophenyl)-4,4-dimethyl-1,4-dihydrobenzo[d][1,3]oxazin-2-one as a yellowish solid (1.4g, 82%): mp 158-159 °C; ^{1}H-NMR (DMSO-d_6) 10.31 (s, 1H, D_2O exchangeable), 7.75 (s, 1H), 7.61 (m, 3H), 7.46 (t, 1H, J= 7.9 Hz), 7.39 (dd, 1H, *J* = 7.0, 1.1 Hz), 6.96 (d, 1H, *J* = 8.6 Hz), 1.68 (s, 6H); Anal. Calc. For $C_{16}H_{14}ClNO_2 \cdot 0.1\ H_2O$: C, 66.37, H, 4.94, N, 4.84. Found: C, 66.14, H, 4.61, N, 4.71.

6-(3-Methoxy-phenyl)-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]-oxazin-2-one (4f). Prepared according to Procedure A from **5** and 3-methoxyphenyl boronic acid. Yellow solid: mp 164-165 °C; ^{1}H-NMR (DMSO-d_6) 10.3 (s, 1H), 7.56 (m, 2H), 7.36 (t, 1 H, *J* = 7.89 Hz), 7.20 (m, 2H), 6.96 (d, 1H, *J* = 8.88 Hz), 6.91 (dd, 1H, *J* = 8.13, 2.35 Hz), 3.8 (s, 3H), 1.7 (s, 6H); MS (ESI) *m/z* 284 ([M+H]$^+$, 30%); Anal. Calc. For $C_{17}H_{17}NO_3 \cdot 1/3H_2O$: C, 70.58, H, 6.11, N, 4.84. Found: C, 70.58, H, 5.73, N, 4.67

6-(2-Chloro-phenyl)-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]oxazin-2-one (4b). Prepared according to Procedure A from **5** and 2-chlorophenyl boronic acid. White solid: mp 181-182 °C; ^{1}H NMR (DMSO-d_6): 10.32 (s, 1H), 7.53-7.58 (m, 1H), 7.32-7.45 (m, 5H), 6.96 (d, 1H, *J* = 8.13 Hz), 1.62 (s, 6H). MS (ESI) *m/z* 288 ([M+H]$^+$, 70%); Anal. Calc. For $C_{16}H_{14}ClNO_2$: C, 66.79, H, 4.90, N, 4.87. Found: C, 66.78, H, 4.82, N, 4.55.

6-(4-Chloro-phenyl)-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]-oxazin-2-one (4d). Prepared according to Procedure A from **5** and 4-chlorophenyl boronic acid. White solid: mp 255-257 °C; ^{1}H-NMR (DMSO-d_6) 10.3 (s, 1H), 7.7 (d, 2H, *J* = 8.52 Hz), 7.55 (m, 2H), 7.5 (d, 2H, *J* = 8.52 Hz), 6.96 (d, 1H, *J* = 8.52 Hz), 1.7 (s, 6H); MS (ESI) *m/z* 288 ([M+H]$^+$,

70%); Anal. Calc. For $C_{16}H_{14}ClNO_2$: C, 66.79, H, 4.90, N, 4.87. Found: C, 66.34, H, 4.76, N, 4.75

3-(4,4-Dimethyl-2-oxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-yl)-benzonitrile (4e, Procedure B). A mixture of **6** (2.22g, 10 mmol), 3-bromobenzonitrile (2.18g, 12 mmol), tetrakis(triphenylphosphine)palladium (0) (0.6g, 0.52 mmol), and sodium carbonate (2.2g, 21 mmol) in a mixture of DME and water (70 mL/15 mL) was degassed to remove the oxygen and then heated at 85 °C under a blanket of nitrogen for 3 hours. The reaction mixture was cooled to ambient temperature and quenched with a saturated aqueous ammonium chloride solution (20 mL). Ethyl acetate (100 mL) was added and organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine and dried with $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by a silica gel flash chromatography (hexane:ethyl acetate/1:1) to give 3-(4,4-dimethyl-2-oxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-yl)-benzonitrile as an off-white solid (0.7g, 25%): mp 236-237 °C; ^{1}H-NMR (DMSO-d_6) 10.34 (s, 1H, D_2O exchangeable), 8.21 (s, 1H), 8.02 (d, 1H, *J* = 8.1 Hz), 7.79 (d, 1H, *J* = 7.7 Hz), 7.60-7.70 (m, 3H), 6.98 (d, 1H, *J* = 8.2 Hz), 1.71 (s, 6H); Anal. Calc. For $C_{17}H_{14}N_2O_2 \cdot 0.1\ H_2O$: C, 72.89, H, 5.11, N, 10.00. Found: C, 72.75, H, 5.05, N, 9.65.

3-(4,4-Dimethyl-2-oxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-yl)-5-fluorobenzonitrile (4h). Prepared from **6** and 3-chloro-5-fluorobenzenenitrile according to procedure B. A white solid (0.7g, 84%): mp 253-254 °C; ^{1}H-NMR (DMSO-d_6) 10.4 (s, 1H, D_2O exchangeable), 8.13 (s, 1H), 7.92 (m, 1H), 7.82 (m, 1H), 7.73 (m, 2H), 6.98 (d, 1H, *J* = 8.2 Hz), 1.68 (s, 6H); ^{19}F-NMR (DMSO-d_6) -112.25 (m, 1F); MS (EI) *m/z* 296 (M^+, 65%); Anal. Calc. For $C_{17}H_{13}FN_2O_2$: C, 68.91, H, 4.42, N, 9.45. Found: C, 68.85, H, 4.58, N, 9.14.

4-(4,4-Dimethyl-2-oxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-yl)-thiophene-2-carbonitrile (4l). Prepared from **6** and 4-bromo-2-thiophenecarbonitrile according to Procedure B. Yellowish solid: mp 230-231 °C (decomposed); ^{1}H-NMR ($CDCl_3$) 8.32 (s, 1H, D_2O exchangeable), 7.83 (d, 1H, *J* = 1.5 Hz), 7.61 (d, 1H, *J* = 1.4 Hz), 7.43 (dd, 1H, *J* = 8.2, 1.9 Hz), 7.29 (d, 1H, *J* = 1.8 Hz), 6.85 (d, 1H, *J* = 8.2 Hz), 1.78 (s, 6H); MS (EI) *m/z*

283(M-H, 100%). Anal. Calc. For $C_{15}H_{12}N_2O_2S \cdot 0.2\ H_2O$: C, 62.57, H, 4.34, N, 9.73. Found: C, 62.48, H, 4.31, N, 9.64.

4-(4,4-Dimethyl-2-oxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-yl)-furan-2-carbonitrile (4m).

Prepared from (1,4-dihydro-4,4-dimethyl-2-oxo-2H-3,1-benzoxazin-6-yl)boronic acid and 4-bromo-2-furancarbonitrile according to Procedure B. Off-white solid: mp 255-256 °C. ^{1}H-NMR (DMSO-d_6) 10.32 (s, 1H, D_2O exchangeable), 8.57 (s, 1H), 8.15 (s, 1H), 7.61 (s, 1H), 7.55 (dd, 1H, *J* = 8.3, 1.5 Hz), 6.92 (d, 1H, *J* = 8.2 Hz), 1.65 (s, 6H); MS (ESI) *m/z* 269(M+H, 72%). Anal. Calc. For $C_{15}H_{12}N_2O_3$: C, 67.16, H, 4.51, N, 10.44. Found: C, 67.14, H, 4.59, N, 10.07.

6-(3-Fluoro-phenyl)-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]-oxazin-2-one (4a).

Prepared from **5** and 1-bromo-3-fluorobenzene according to Procedure A. A light yellow solid: mp 181-182 °C; ^{1}H-NMR (DMSO-d_6) 10.4 (s, 1H), 7.62-7.44 (m, 5H), 7.16 (t, 1H, *J* = 2.22 Hz), 6.97 (d, 1H, *J* = 8.83), 1.67 (s, 6H); MS (EI) *m/z* 271 ([M + H]$^+$, 40%); Anal. Calc. For $C_{16}H_{14}FNO_2$: C, 69.91, H, 5.3, N, 5.1. Found: C, 70.0, H, 5.32, N, 4.92.

6-(3-Chloro-4-fluoro-phenyl)-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]oxazin-2-one (4j).

Prepared from **6** and 1-bromo-3-chloro-4-fluorobenzene according to Procedure B. A white solid: mp 211-212 °C; ^{1}H-NMR (DMSO-d_6) 10.4 (s, 1H), 7.92 (dd, 1H, *J* = 7.13, 2.19 Hz), 7.71-7.66 (m, 1H), 7.60-7.57 (m, 2H), 7.49 (t, 1H, *J* = 8.95 Hz), 6.96 (d, 1H, *J* = 8.01 Hz), 1.67 (s, 6H); MS (EI) *m/z* 305 ([M + H]$^+$, 20%); Anal. Calc. For $C_{16}H_{13}ClFNO_2$: C, 62.86, H, 4.29, N, 4.58. Found: C, 62.52, H, 4.45, N, 4.42.

6-(3-Chloro-5-fluoro-phenyl)-4,4-dimethyl-1,4-dihydro-benzo[d][1,3]oxazin-2-one (4g).

Prepared from **6** and 1-bromo-3-chloro-5-fluorobenzene according to procedure B. A white solid: mp 193-194 °C; ^{1}H-NMR (DMSO-d_6) 10.4 (s, 1H), 7.67-7.64 (m, 3H), 7.61-7.57 (m, 1H), 7.41-7.37 (m, 1H), 6.96 (d, 1H, *J* = 8.72 Hz), 1.7 (s, 6H); MS (APCI) *m/z* 306([M + H]$^+$, 100%); Anal. Calc. For $C_{16}H_{13}ClFNO_2$: C, 62.86, H, 4.29, N, 4.58. Found: C, 62.98, H, 4.1, N, 4.6.

5-(4,4-Dimethyl-2-oxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-yl)-2-fluoro-benzonitrile (4i). Prepared from **6** and 1-bromo-2-fluorobenzonitrile according to procedure B. A white solid: mp 255-256 °C; ^{1}H-NMR (DMSO-d_6) 10.4 (s, 1H), 8.30 (dd, 1H, *J* = 6.15, 2.41 Hz), 8.12-8.07 (m, 1H), 7.76-7.58 (m, 3H), 6.97 (d, 1H, *J* = 8.22 Hz), 1.7 (s, 6H); MS (APCI) *m/z* 297 ([M+H]$^+$, 100%); Anal. Calc. For $C_{17}H_{13}FN_2O_2 \cdot 0.1\ H_2O$: C, 68.50, H, 4.46, N, 9.40. Found: C, 68.27, H, 4.81, N, 9.1.

3-Fluoro-5-(8-fluoro-4,4-dimethyl-2-oxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-yl)-benzonitrile (4k). Prepared from 8-fluoro-(1,4-dihydro-4,4-dimethyl-2-oxo-2H-3,1-benzoxin-6-yl)boronic acid and 5-bromo-3-fluorobenzonitrile according to procedure B**.** A white solid: mp 256-257 °C; ^{1}H-NMR (DMSO-d_6) 10.5 (s, 1H), 8.20 (bs, 1H), 8.06 (dt, 1H, *J* = 10.48, 2.16 Hz), 7.85-7.82 (m, 1H), 7.77 (dd, 1H, *J* = 11.89, 1.81 Hz), 7.63 (s, 1H), 1.7 (s, 6H); MS (EI) *m/z* 314([M$^+$], 60%). Anal. calcd for $C_{17}H_{12}F_2N_2O_2 \cdot 0.1H_2O$: C, 64.54, H, 3.86, N, 8.86. Found: C, 64.22, H, 3.80, N, 8.50.

Rat decidualization assay

A. Reagents

Test compounds were dissolved in 100% ethanol and mixed with corn oil (vehicle). Stock solutions of the test compounds in oil (Mazola^{TM}) were then prepared by heating (~80 ^{O}C) the mixture to evaporate ethanol. Test compounds were subsequently diluted with 100% corn oil or 10% ethanol in corn oil prior to the treatment of animals. No difference in decidual response was found when these two vehicles were compared.

B. Animals

Ovariectomized mature female Sprague-Dawley rats (~60-day old and 230g) were obtained from Taconic (Taconic Farms, NY) following surgery. Ovariectomy was performed at least 10 days prior to treatment to reduce circulating sex steroids. Animals were housed under 12 hr light/dark cycle and given standard rat chow and water *ad libitum.*

C. Treatment

Rats were weighed and randomly assigned to groups of 4 or 5 before treatment. Test compounds in 0.2 ml vehicle were administered by subcutaneous injection in the nape of the neck or by gavage using 0.5 ml. The animals were treated once daily for seven days. For testing antiprogestins, animals were given the test compounds and an EC_{50} dose of progesterone (5.6 mg/kg) during the first three days of treatment. Following decidual stimulation, animals continued to receive progesterone until necropsy four days later.

D. Dosing*:*

Doses were prepared based upon mg/kg mean group body weight. In all studies, a control group receiving vehicle was included. Determination of dose-response curves was carried out using doses with half log increases (e.g. 0.1, 0.3, 1.0, 3.0 mg/kg...).

E. Decidual induction**:**

Approximately 24 hr after the third injection, decidualization was induced in one of the uterine horns by scratching the antimesometrial luminal epithelium with a blunt 21 G needle. The contralateral horn was not scratched and served as an unstimulated control. Approximately 24 hr following the final treatment, rats were sacrificed by CO_2 asphyxiation and body weight measured. Uteri were removed and trimmed of fat. Decidualized (D-horn) and control (C-horn) uterine horns were weighed separately.

F. Analysis of Results:

The increase in weight of the decidualized uterine horn was calculated by D-horn/C-horn and logarithmic transformation was used to maximize normality and homogeneity of variance. The Huber M-estimator was used to down weight the outlying transformed observations for both dose-response curve fitting and one-way analysis of variance. JMP software (SAS Institute, Inc.) was used for both one-way ANOVA and non-linear dose-response analyses.

Rat uterine C3 model

A. Reagents

Stock solutions of the test compounds are prepared in 100% ethanol or 100% DMSO if they are not soluble in ethanol. The compounds are prepared for dosing in 10% ethanol in corn oil (Mazola^{TM}) vehicle.

B. Animals

Ovariectomized-female, 60 day-old Sprague-Dawley rats are obtained following surgery (Harlan). The surgeries are to be done a minimum of 8 days prior to the first treatment. The animals are housed under 12 hr. light/dark cycle. In some of the assay validation studies, the animals were fed standard rat chow and water ad libitum, while in others the animals were fed the casein-based Laboratory Rodent Diet 5K96 (Purina) and water ad libitum. All future studies will be run using the 5K96 diet.

C. Treatment

Upon arrival the rats are randomized, placed in groups of six, and given a minimum of 72 hours to acclimate to the surroundings. They are then treated once a day for two days with the compound(s) of interest or vehicle (vehicle control group). Administration of the compound is either by subcutaneous injection of 0.2 ml in the nape of the neck or orally by gavage of 0.5 ml. On the second day, the animals are co-treated with 17 -ethinyl estradiol (EE) or vehicle (vehicle control group), orally by gavage, 0.5 ml per dose Dosing: Two control groups are included in all analyses: a vehicle group and an EE group. Doses are prepared based on mg/kg mean group body weight. Initial screening of test compounds is done at three doses (e.g. 0.03, 0.3, 3 mg/kg body weight). Dose-response curves may be run on active compounds using doses with half-log increments over a dose range determined from the initial screening data. Approximately 24 hours following the final treatment the animals are killed by CO2 asphyxiation and the body weight and uterine wet weight determined.

D. Assays uterine complement component C3

Following euthanasia, the uteri are removed from the animals, stripped of remaining fat and mesentary and weighed. The uteri are snap-frozen on dry ice in groups of two. Total RNA is extracted using TRIzol reagent (GIBCO BRL). RNA samples are run on a 1% agarose/formaldehyde gel. The nucleic acids are then transferred to a nylon membrane

overnight by capillary action. The nucleic acids are crosslinked to the membrane using UV light, the 28S rRNA is quantitated using the IP Lab Gel software (Signal Analytics Corp.), and then the blot is hybridized with a cDNA probe for complement component C3. Following hybridization, the blot is exposed to a phosphorscreen. Quantitation of C3 message is performed using a phosphorimager (Molecular Dynamics).

F. Analysis of Results

Results are reported as the ratio C3/28S. These ratios are transformed by logarithms to normalize the data. The Huber M-estimator is used to down weight the outlying transformed observations. The JMP software (SAS Institute, Inc.) is used to analyze the transformed and weighted data for both the one-way ANOVA and the non-linear dose response curves. The IC50 values with 95% confidence intervals are calculated using a 4 parameter logistic model that calculates minimum, maximum, slope, and IC50.