Supplemental Information

Substituted 6-(1-Pyrrolidine)quinolin-2(1H)-ones As Novel Selective Androgen Receptor Modulators

Esther Martinborough, Yixing Shen, Arjan van Oeveren, Yun Oliver Long, Thomas L.S. Lau, Keith B. Marschke, William Y. Chang, Francisco J. López, Eric G. Vajda, Peter J. Rix, O. Humberto Viveros, Andrés Negro-Vilar, Lin Zhi

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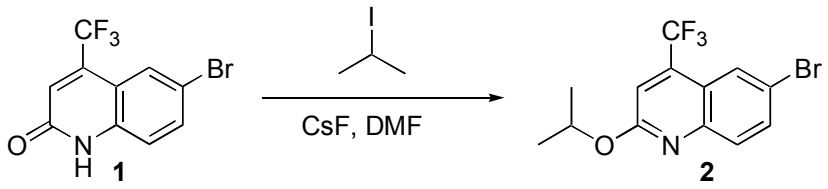
General Experimental Methods.

Melting points were determined with an Electrothermal IA9100 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 500 MHz on a Varian VXR-500 or at 400 MHz on a Bruker AC400. Chemical shifts are given in ppm relative to internal tetramethylsilane or solvent. Coupling constants (*J*) are given in Hertz (Hz). HRMS were recorded on a Micromass LCT mass spectrometer. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ. Reagents and solvents were obtained from commercial sources and used without further purification. Reported yields are not optimized.

Experimentals

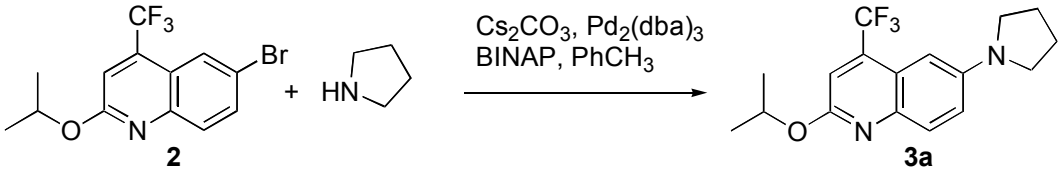


6-Bromo-2-isopropoxy-4-trifluoromethylquinoline 2.

CsF (65.3g, 0.43mol) was added to a solution of quinolin-2(1*H*)-one **1** (29.2g, 0.10mol) in dry DMF (400mL) followed by 2-Iodopropane (32mL, 0.32mol) under a nitrogen atmosphere. It was stirred at room temperature for 18h. The mixture was poured into water (500mL) and extracted with EtOAc (3× 300mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification by chromatography (silica gel, hexane/EtOAc 10:1) yielded bromide **2** (32.6g, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) : δ 8.12 (d, 1H, *J*= 1.0), 7.75 (s, 2H), 7.20 (s, 1H), 5.56 (dq, 1H, *J*= 6.3, 6.3), 1.43 (d, 6H, *J*=5.9).

Palladium catalyzed amination of bromide 2, general procedure

To a degassed solution of the bromide **2** (5.0g, 15mmol) in 30mL toluene was added Cs_2CO_3, (10g, 28mmol), Pd_2dba_3 (0.28g, 0.30mmol), *rac*-BINAP (0.28g, 0.45mmol), and the pyrrolidine (20mmol) or pyrrolidin-2-one. The heterogeneous mixture was heated at reflux until completion of the reaction by TLC, cooled to rt and water (100mL) was added. The mixture was extracted with EtOAc (2× 100mL) and the combined organic layers washed with brine and dried over $MgSO_4$. Purification by chromatography (silica gel, hexane/ CH_2Cl_2) afforded the product.

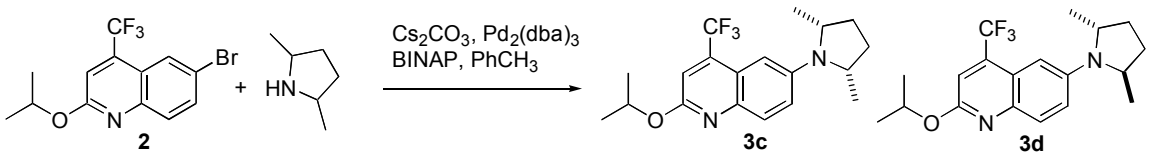


2-Isopropoxy-6-pyrrolidin-1-yl-4-trifluoromethylquinoline 3a.

Prepared from bromide **2** (1.0g, 3.0mmol) and 0.8g pyrrolidine (11mmol). Refluxed for 20 h. Purification by chromatography (silica gel, hexane/ CH_2Cl_2 4:1 to 1:2 gradient) yielded 0.9g of **3a**. ^{1}H NMR(500 MHz, $CDCl_3$) : δ 7.73 (d,1H, *J*= 9.3), 7.15 (dd, 1H, *J*=9.3, 2.4), 7.01 (s, 1H), 6.87-6.85 (m, 1H), 5.49 (dq, 1H, *J*=5.9, 6.3), 3.41-3.39 (m, 4H), 2.08-2.06 (m, 4H), 1.40 (d, 6H, *J*=5.9).

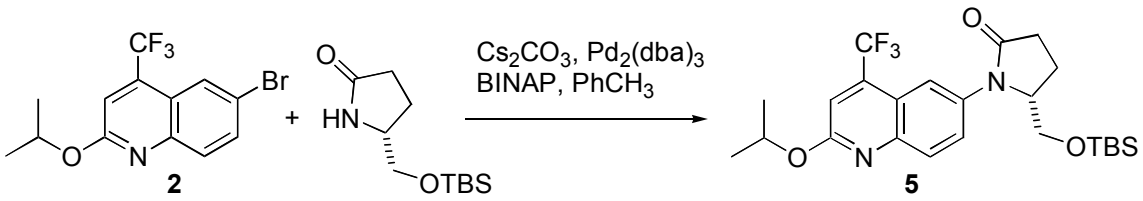
2-Isopropoxy-6-(2-methylpyrrolidin-1-yl)-4-trifluoromethylquinoline 3b.

Prepared from 33.4 mg bromide **2** (0.1mmol) and 0.1mL 2-methylpyrrolidine (0.8mmol). Refluxed for 20h. Purification by chromatography (silica gel, hexane/ CH_2Cl_2 4:1 to 1:2 gradient) yielded 7.5mg of **3b** (22%). ^{1}H NMR(500 MHz, $CDCl_3$) : δ 7.73 (d,1H, *J*= 9.3), 7.17 (dd, 1H, *J*=9.3, 2.9), 7.09 (m, 1H), 6.87-6.86 (m, 1H), 5.49 (dq, 1H, *J*=5.9, 6.3), 4.02-4.00 (m,1H), 3.56-3.52 (m, 1H), 3.27-3.12 (m, 1H), 2.16-2.11 (m, 2H), 2.09-2.03 (m, 1H), 1.78-1.71 (m, 1H), 1.39 (d, 6H, *J*=5.9), 1.24 (d, 3H, *J*=6.3)



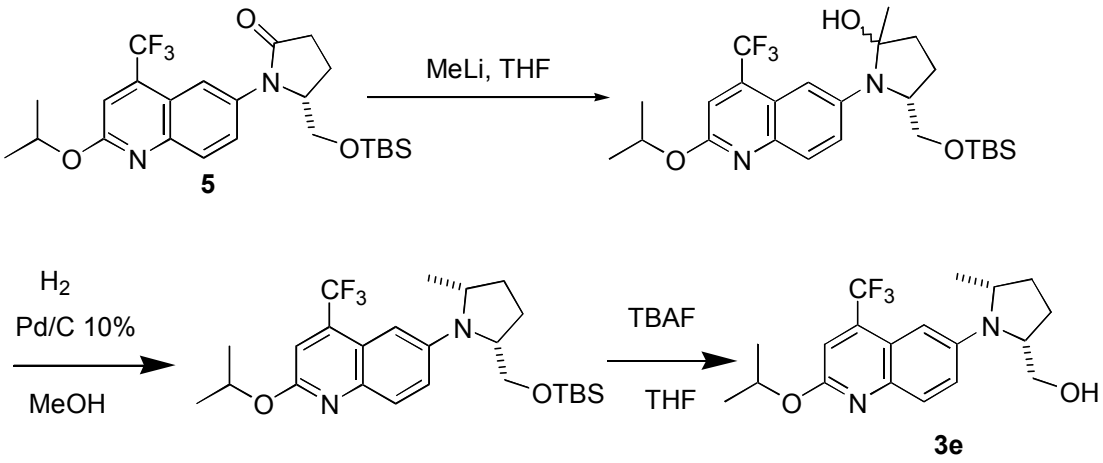
6-(*cis*-2,5-Dimethyl-pyrrolidin-1-yl)-2-isopropoxy-4-trifluoromethylquinoline 3c and 6-(*rac*, *trans*-2,5-dimethylpyrrolidin-1-yl)-2-isopropoxy-4-trifluoromethylquinoline 3d. Prepared from 1.0g bromide

(3.0mmol) and 1.0g 2,5-dimethylpyrrolidine (10mmol). Refluxed for 20 h. Purification by chromatography (silica gel, hexane/ CH_2Cl_2 6:1 to 1:2 gradient) yielded 0.45g (44%) **3c**. ^{1}H NMR(500 MHz, $CDCl_3$) : δ 7.73 (d,1H, *J*= 9.3), 7.20 (dd, 1H, *J*=9.3, 2.4), 7.09 (m, 1H), 6.88-6.85 (m, 1H), 5.49 (dq, 1H, *J*=5.9, 6.3), 3.91-3.88 (m,2H), 2.15-2.10 (m, 2H), 1.80-1.76 (m, 2H), 1.39 (d, 6H, *J*=5.9), 1.34 (d, 6H, *J*=6.3), and 0.01 g **3d** ^{1}H NMR(400 MHz, $CDCl_3$) : δ 7.71 (d,1H, *J*= 9.2), 7.17 (dd, 1H, *J*=9.2, 2.6), 7.08 (m, 1H), 6.86 (s, 1H), 5.48 (dq, 1H, *J*=5.9, 6.3), 4.14-4.10 (m,2H), 2.30-2.26 (m, 2H), 1.71-1.68 (m, 2H), 1.38 (d, 6H, *J*=6.1), 1.15 (d, 6H, *J*=6.1).

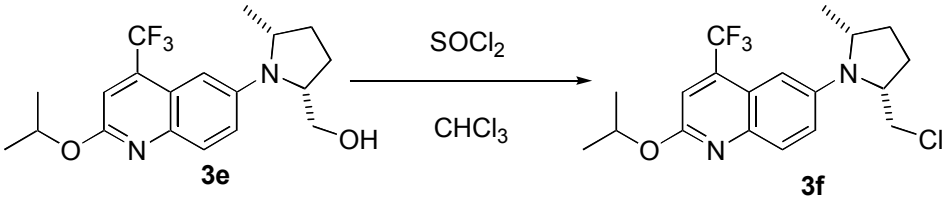


(*R*)-5-(*tert*-Butyldimethylsilanyloxymethyl)-1-(2-isopropoxy-4-trifluoromethylquinolin-6-yl)-pyrrolidin-2-one 5.

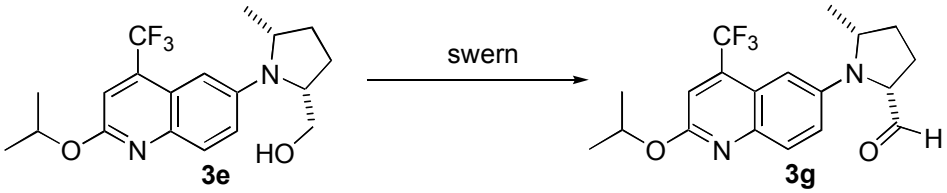
Cesium carbonate (22.8g, 64.6mmol) was added to the bromide **2** (6.36g, 19mmol) in toluene (35mL) followed by $Pd_2(dba)_3$ (522mg, 0.57mmol), racemic BINAP (1.06g, 1.71mmol) and (*R*)-5-(*tert*-butyl-dimethylsilanyloxymethyl)-pyrrolidin-2-one (8.72g, 38mmol). The reaction was immediately evacuated-purged with N_2 twice, and heated to reflux for 26h. The mixture was poured into water and extracted with EtOAc. The combined organic extracts were concentrated under reduced pressure and purified by flash chromatography (Silica gel, hexanes/EtOAc 1:0 to 4:1 gradient) to yield TBS alcohol **5** (7.26g, 80%). ^{1}H NMR(500 MHz, $CDCl_3$) : δ 7.73 (d,1H, *J*= 9.3), 7.20 (dd, 1H, *J*=9.3, 2.4), 7.09 (bs, 1H), 6.88-6.85 (m, 1H), 5.49 (dq, 1H, *J*=5.9, 6.3), 3. (m,2H), 2. (m, 2H), 1. (m, 2H), 1.39 (d, 6H, *J*=5.9), 1.34 (s, 9H), 0.20 (s, 6H)



[(2*R*,5*R*)-1-(2-Isopropoxy-4-trifluoromethylquinolin-6-yl)-5-methylpyrrolidin-2-yl]-methanol 3e. 1.4 M methyl lithium in diethyl ether (40mL, 56mmol) was added slowly to a solution of TBS alcohol **5** (8.83g, 0.0183mol) in dry THF (80mL) under a nitrogen atmosphere at -78°C. The reaction was stirred at -78°C for an additional 4 h. MeOH was added slowly at -78°C. It was filtered through silica gel and concentrated under reduced pressure to yield a mixture of crude hemi-aminals. This mixture was dissolved in 10%TFA/MeOH (120mL), and 10% Palladium on carbon (0.88g) was added. The suspension was shaken in a Parr-Shaker for 84h under a 50psi hydrogen atmosphere. The suspension was filtered through silica gel and washed with MeOH and the filtrate concentrated under reduced pressure to give the TBS protected alcohol. This was dissolved in 50mL THF and 1.0M TBAF (64mL, 64mmol) was added. The mixture was stirred at room temperature for 18h. poured into water (200mL) and extracted with 2×200mL EtOAc. The combined organic extracts were concentrated under reduced pressure and purified by flash chromatography (Silica gel, hexane/EtOAc 5:1 to 2:1 gradient) to yield alcohol **3e** (5.55g, 82% over 3 steps) ^{1}H NMR(500 MHz, $CDCl_3$) : δ 7.75 (d,1H, *J*= 9.3), 7.33 (dd, 1H, *J*=9.3, 2.4), 7.12 (s, 1H), 7.07 (bs, 1H), 5.50 (dq, 1H, *J*=5.9, 6.3), 4.00-3.96 (m,1H), 3.93-3.89 (m, 1H), 3.82-3.79 (m, 1H), 3.74-3.70 (m, 1H), 2.20-2.13 (m, 1H), 2.10-2.01 (m, 2H), 1.81-1.76 (m 1H), 1.69 (bs, 1H), 1.39 (d, 6H, *J*=5.9), 1.37 (d, 3H, *J*=6.3)

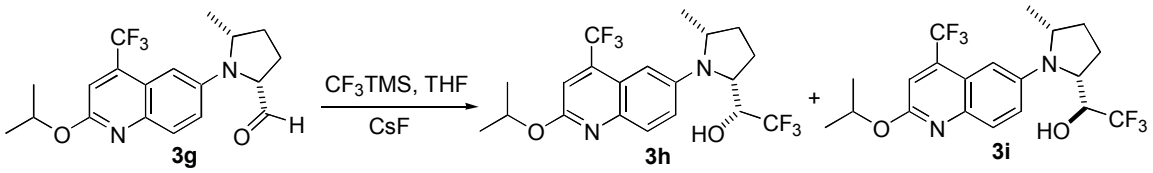


6-((2*R*,5*R*)-2-Chloromethyl-5-methylpyrrolidin-1-yl)-2-isopropoxy-4-trifluoromethylquinoline 3f. To a solution of the alcohol **3e** (2.0g, 5.4mmol) in 25 mL $CHCl_3$ was added $SOCl_2$ (5mL, 0.07 mol) and the mixture was heated at rt for 1h, and then at 40°C for 3h. The mixture was cooled to rt and sat $NaHCO_3$ (50mL) was added. The mixture was extracted with EtOAc (100mL), the organic layer was washed with brine and dried over $MgSO_4$. The product was purified by chromatography (silica gel, hexane, CH_2Cl_2 6:1 to 1:1 gradient) to give **3f** (1.3g, 65%). ^{1}H NMR(500 MHz, $CDCl_3$) : δ 7.77 (d,1H, *J*= 9.3), 7.20 (dd, 1H, *J*=9.3, 2.9), 7.13 (s, 1H), 6.98 (s, 1H), 5.51 (dq, 1H, *J*=5.9, 6.3), 4.07-4.02 (m, 1H), 3.95-3.91 (m, 1H), 3.79 (dd, 1H, *J*=10.8, 2.9), 3.40 (t, 1H, *J*=10.8), 2.28-2.21 (m, 1H), 2.16-2.11 (m, 2H), 1.83-1.76 (m, 1H), 1.41 (d, 6H, *J*=6.3), 1.36 (d, 3H, *J*=5.9)



(2*R*,5*R*)-1-(2-Isopropoxy-4-trifluoromethylquinolin-6-yl)-5-methylpyrrolidine-2-carbaldehyde 3g. 2.0 M Oxalyl chloride in CH_2Cl_2 (2.65ml, 5.3mmol) was added to DMSO (0.375mL, 5.3mmol) in CH_2Cl_2 (20mL) at -78 °C. and the mixture was stirred at this temperature for 30 min. Alcohol **3e** (1.5g, 4.1mmol) in CH_2Cl_2 (30mL) was added and the mixture was stirred at -78 °C for 2 h. Triethylamine (5.7mL, 41mmol) was added and the mixture was allowed to warm to room temperature. The mixture was poured into 100mL water and extracted with 2×100mL EtOAc. The combined organic layers were washed with brine and dried over $MgSO_4$ Approximately 200mL hexane was added and the mixture was filtered through a short plug of silica gel, and rinsed with EtOAc/hexane 1:1. Concentration afforded **3g**(1.39g, 93%).^{1}H NMR (500 MHz, $CDCl_3$) : δ 9.51 (d, 1H, *J*=3.9), 7.74 (d,1H, *J*= 9.3), 7.12 (s, 1H), 7.10 (dd, 1H, *J*=9.3, 2.9), 6.97 (s, 1H),

5.49 (dq, 1H, *J*=5.9, 6.3), 4.16-4.10 (m, 2H), 2.25-2.17 (m, 3H), 1.81-1.76 (m, 1H), 1.41 (d, 3H, *J*=6.3), 1.39 (d, 6H, *J*=6.3)



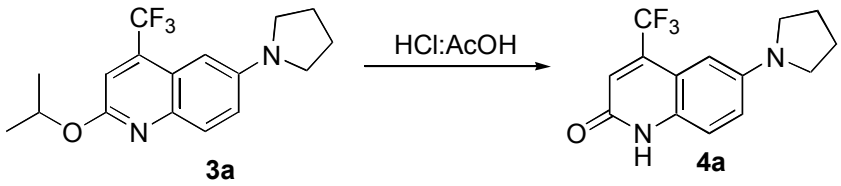
(*R*)-2,2,2-Trifluoro-1-[(2*R*,5*R*)-1-(2-isopropoxy-4-trifluoromethylquinolin-6-yl)-5-methylpyrrolidin-2-yl]ethanol 3h and **(*S*)-2,2,2-Trifluoro-1-[(2*R*,5*R*)-1-(2-isopropoxy-4-trifluoromethylquinolin-6-yl)-5-methylpyrrolidin-2-yl]ethanol 3i**.

Trifluoromethyltrimethylsilane (0.86mL, 5.5mmol) was added to a solution of aldehyde **3g** (1.39g, 3.8mmol) in THF (15 mL) and CsF (3.09g, 20.4mmol) at - 78 °C. The cooling bath was removed and the mixture was allowed to warm to room temperature and stirred for 18h. Ethanol (15mL) was added and the reaction stirred at room temperature for an additional 3h. The mixture was poured into 100mL water and extracted with 2×100mL ethyl acetate. The combined organic extracts were concentrated under reduced pressure and purified by chromatography (silica gel, hexane/CH_2Cl_2) to give alcohol **3h** (0.512g, 31%) and diastereomer **3i** (0.795g, 48%). **3h** ^{1}H NMR (500 MHz, $CDCl_3$) 7.77 (d, 1H, *J*=9.5), 7.26 (dd, 1H, *J*=9.5, 2.5), 7.13 (s, 1H), 7.00 (s, 1H), 5.50 (m, 1H), 4.45 (m, 1H), 4.19 (m, 1H), 3.94 (m, 1H), 2.48 (m, 2H), 2.08(m, 2H), 1.83 (m, 1H), 1.40 (m, 9H)

3i ^{1}H NMR (500 MHz, $CDCl_3$) 7.79 (d, 1H, *J*=9.5), 7.40 (dd, 1H, *J*=9.5, 3.0), 7.31(bs, 1H), 7.16 (s, 1H), 5.54 (m, 1H), 4.13 (m, 2H), 3.89 (m, 1H), 3.73 (m, 1H), 3.42 (s, 1H), 2.33 (m, 1H), 2.17 (m, 1H), 1.92 (m, 1H) , 1.76(m, 1H), 1.40(m, 9H)

Deprotection of 2-isopropoxyquinolines, general procedure

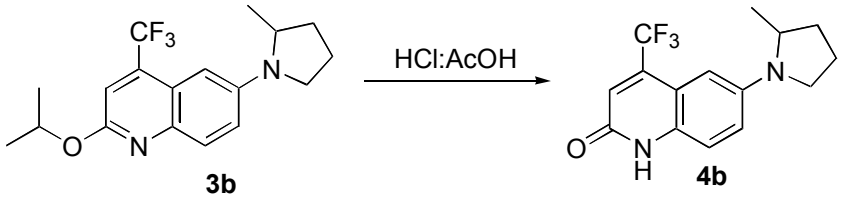
A mixture of the 2-isopropoxyquinoline **3** (5mmol) in 8mL HOAc and 2mL conc HCl was heated at 60°C until completion of the reaction by TLC. Water was added and the mixture was neutralized by addition of 20% NaOH. Extracted with EtOAc, organic layer washed with brine and dried over $MgSO_4$. Concentrated and purified by chromatography.



3a **4a**

6-Pyrrolidin-1-yl-4-trifluoromethyl-1*H*-quinolin-2-one 4a

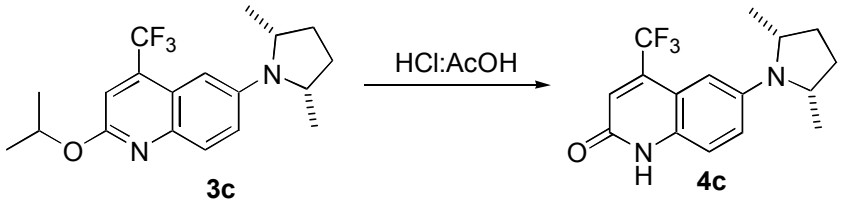
Prepared from alcohol **3a** (29mg, 0.09mmol). Purification by chromatography (silica gel, CH_2Cl_2/MeOH 9:1) afforded **4a** (26mg, 100%) as a yellow solid. ^{1}H NMR(500 MHz, acetone-d_6) : δ 10.4 (bs, 1H), 7.41 (d,1H, *J*= 9.3), 7.07 (dd, 1H, *J*=9.3, 2.9), 6.87 (s, 1H), 6.76 (t, 1H, *J*=2.4), 3.34-3.31 (m, 4H), 2.08-2.04 (m, 4H)



3b **4b**

***rac*-6-(2-Methylpyrrolidin-1-yl)-4-trifluoromethyl-1*H*-quinolin-2-one 4b**

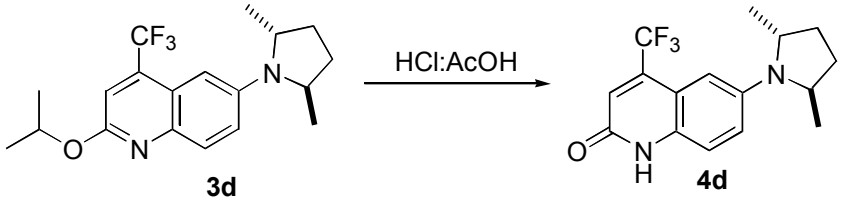
Prepared from **3b** (7.5mg, 0.02mmol). Purification by chromatography (silica gel, CH_2Cl_2/MeOH 9:1 to 6:1 gradient) afforded **4b** (6.6mg, 100%) as a yellow solid. ^{1}H NMR(500 MHz, acetone-d_6) : δ 11.8 (bs, 1H), 7.31 (d,1H, *J*= 9.3), 7.06 (bs, 1H), 6.99 (dd, 1H, *J*=9.3, 2.4), 6.83 (bs, 1H), 3.94-3.91 (m, 4H), 3.48 (dt, 1H, *J*=9.8, 2.9), 3.22 (q, 1H, *J*=7.3), 2.16-2.10 (m, 2H), 2.10-2.02 (m, 1H), 1.77-1.76 (m, 1H), 1.21 (d, 3H, *J*=6.3); HPLC method **A**: Beckman, Si dp (5μM), Hexane/Ethanol 85:15, flow rate 1 mL/min, retention time 5.1 min, 97.5% purity; method **B**: Kromasil 100 C18 (5μM), Methanol/water 60:40, flow rate 1 mL/min, retention time 13.3 min, 98.2% purity.



3c **4c**

6-(*cis*-2,5-Dimethylpyrrolidin-1-yl)-4-trifluoromethyl-1*H*-quinolin-2-one 4c

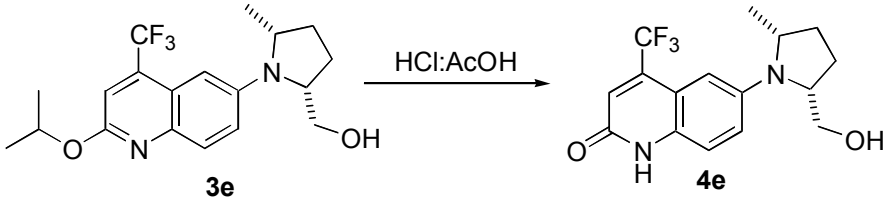
Prepared from **3c** (11.7mg, 0.03mmol). Purification by chromatography (silica gel, CH_2Cl_2/MeOH 9:1 to 6:1 gradient) afforded **4c** (10.7mg, 100%) as a yellow solid. ^{1}H NMR(500 MHz, $CDCl_3$) : δ 11.6 (bs, 1H), 7.29 (d,1H, *J*= 9.3), 7.05 (bs, 1H), 7.02 (dd, 1H, *J*=9.3, 2.4), 6.88 (bs, 1H), 3.82-3.78 (m, 2H), 2.14-2.08 (m, 2H), 1.80-1.75 (m, 2H), 1.31 (d, 6H, *J*=6.3)



3d **4d**

***rac*-6-(*trans*-2,5-Dimethylpyrrolidin-1-yl)-4-trifluoromethyl-1*H*-quinolin-2-one 4d**

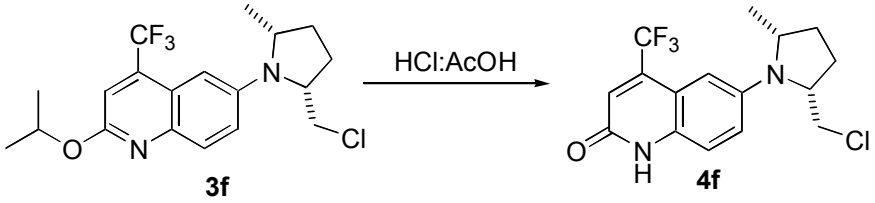
Prepared from **3d** (1.5mg, 0.01mmol). Purification by chromatography (silica gel, CH_2Cl_2/MeOH 9:1 to 6:1 gradient) afforded **4d** (1.3mg, 100%) as a yellow solid. ^{1}H NMR(500 MHz, $CDCl_3$) : δ 11.3 (bs, 1H), 7.26 (d,1H, *J*= 8.8), 7.04 (bs, 1H), 6.99 (dd, 1H, *J*=9.3, 2.9), 6.83 (bs, 1H), 4.05 (t, 2H, *J*=6.3), 2.30-2.27 (m, 2H), 1.70 (d, 2H, *J*=5.4), 1.13 (d, 6H, *J*=6.3)



3e **4e**

6-((2*R*,5*R*)-2-Hydroxymethyl-5-methylpyrrolidin-1-yl)-4-trifluoromethyl-1*H*-quinolin-2-one 4e

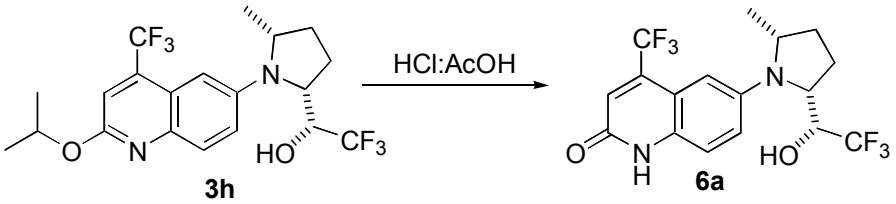
Prepared from alcohol **3e** (6.0mg, 0.016mmol). Purification by chromatography (silica gel, CH_2Cl_2/MeOH 9:1 to 6:1 gradient) afforded **4e** (4.9mg, 92%) as a yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$) 11.80 (bs, 1H), 7.31 (d, 1H, *J*=9.2), 7.18 (d, 1H, *J*=8.9), 7.06 (s, 1H), 7.03 (s, 1H), 3.90-3.69 (m, 4H), 1.78-1.44 (m, 4H), 1.34 (d, 3H, *J* = 6.1)



3f **4f**

6-((2*R*,5*R*)-2-Chloromethyl-5-methylpyrrolidin-1-yl)-4-trifluoromethyl-1*H*-quinolin-2-one 4f

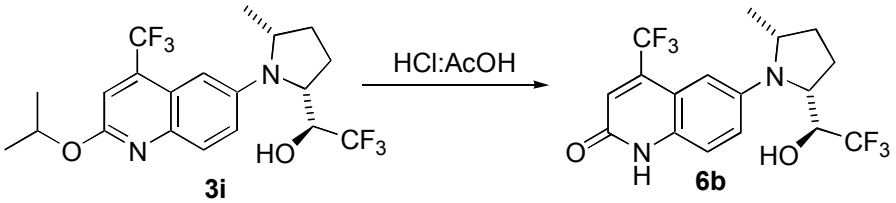
Prepared from **3f** (0.40g, 1.1mmol). Purification by chromatography (silica gel, CH_2Cl_2/MeOH 9:1 to 6:1 gradient) afforded **4f** (0.23g, 64%) as a yellow solid. **^{1}H NMR** (500 MHz, CD_3OD) 7.41 (d, 1H, *J*=8.8), 7.23 (dd, 1H, *J*=9.3, 2.4), 7.01 (s, 1H), 6.95 (s, 1H), 4.00-3.95 (m, 1H), 3.90-3.87 (m, 1H), 3.77 (dd, 1H, *J*= 10.7, 2.9), 3.53 (dd, 1H, *J*=10.7, 9.3), 2.29-2.22 (m, 1H), 2.17-2.05 (m, 2H), 1.86 (m, 1H), 1.35 (d, 3H, *J* = 6.3)



3h **6a**

6-[(2*R*,5*R*)-2-Methyl-5-((*R*)-2,2,2-trifluoro-1-hydroxyethyl)pyrrolidin-1-yl]-4-trifluoromethyl-1*H*-quinolin-2-one 6a.

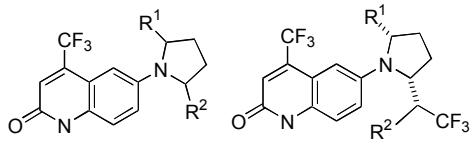
Prepared from alcohol **3h** (1.35g, 3.1mmol). Purification by chromatography (silica gel, CH_2Cl_2/MeOH 9:1 to 6:1 gradient) afforded **6a** (1.01g, 83%) as a yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$) 12.20 (br, 1H), 7.33 (d, 1H, *J*=9.5), 7.08 (dd, 1H, *J*=9.5, 2.5), 6.98 (s, 1H), 6.84 (m, 1H), 4.39 (m, 1H), 4.09 (m, 1H), 3.82 (m, 1H), 3.42 (br, 1H), 2.49 (m, 1H), 2.01-2.09 (m, 2H), 1.78 (m, 1H), 1.32 (d, 3H, *J* = 6.5)



6-[(2*R*,5*R*)-2-Methyl-5-((*S*)-2,2,2-trifluoro-1-hydroxyethyl)pyrrolidin-1-yl]-4-trifluoromethyl-1*H*-quinolin-2-one 6b.

Prepared from alcohol **3i** (0.51g, 1.2mmol). Purification by chromatography (silica gel, CH_2Cl_2/MeOH 9:1 to 6:1 gradient) afforded **6b** (0.41g, 90%) as a yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$) 11.59 (br, 1H), 7.35 (d, 1H, *J*=9.0), 7.28 (dd, 1H, *J*=9.5, 3.0), 7.25 (m, 1H), 7.08 (s, 1H), 4.04 (t, 1H, *J* = 7.5), 3.79 (m, 1H), 3.71 (m, 1H), 3.45 (s, 1H), 2.32 (m, 1H), 2.05 (m, 1H), 1.91(m, 1H), 1.72 (m, 1H), 1.40 (d, 3H, *J*=6.0)

Table S-1 Analytical data for substituted 6-(pyrrolidine)-4-trifluoromethylquinolin-2(*1H*)-ones.



4 **6**

No	R_1	R_2	formula	Mp (^oC)	HRMS M+H (theor)	%C (theor)	%H (theor)	%N (theor)
4a	H	H	$C_{14}H_{13}F_3N_2O$	246-248	253.2058 (253.1059)	-	-	-
4b	H	Me	$C_{15}H_{15}F_3N_2O$	156-158	297.1216 (297.1216)	-	-	-
4c	Me	Me	$C_{16}H_{17}F_3N_2O$	183-184	311.1369 (311.1372)	61.91(61.93)	5.44(5.52)	8.94(9.03)
4d	Me	Me	$C_{16}H_{17}F_3N_2O$	221-223	-	61.67(61.93)	5.50(5.52)	8.89(9.03)
4e	Me	CH_2OH	$C_{16}H_{17}F_3N_2O_2$	138-139	-	57.23 (58.89)	4.99(5.25)	8.40(8.58)
4f	Me	CH_2Cl	$C_{16}H_{16}ClF_3N_2O$	179-180	345.0980 (345.0982)	55.52(55.74)	4.72(4.68)	7.86(8.13)
6a	Me	(*R*)-OH	$C_{17}H_{16}F_6N_2O_2$	246-248	395.1199 (395.1195)	52.00(51.78)	4.01(4.09)	7.05(7.10)
6b	Me	(*S*)-OH	$C_{17}H_{16}F_6N_2O_2$	243-244	395.1198 (395.1195)	51.43(51.78)	3.97(4.09)	6.81(7.10)

Biological Methods
A description of the in vitro assays and the 2 week ORDX rat model can be found in references 1 and 2. [1,2]

PK Assay
Intravenous Dosing
Adult male Sprague –Dawley rats received a single tail vein injection of 4 ml/kg of the test sample intravenous dosing solution (vehicle: DMSO:serum (10:90), nominal dose was 2.5 mg/kg). A single blood sample (~ 250 µl) was taken from the jugular vein cannula at each of 5, 10, 20, 40 min, and 1, 2, 4, 6, 8 and 12 hr post-dose using a 1 ml tuberculin syringe. Immediately following blood sampling, the withdrawn blood volume was replaced with heparinized saline. The blood samples were immediately transferred into EDTA-treated tubes. Blood samples were stored in a 4°C refrigerator until they could be processed, generally within one hour. Plasma was separated from whole blood by centrifugation and stored at –20°C until analysis.

Oral Dosing
Animals in the single oral dosing groups received 10 ml/kg of the oral dosing suspension (vehicle: polyethylene glycol 400: Tween 80: Polyvinylpyrrolidone K30: water (8.96:0.52:0.52:90). Animals were fasted for at least 12 hours prior to dose administration. A single blood sample (~ 250 µl) was taken from the jugular vein cannula at each of 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 hr post-dose using a 1 ml tuberculin syringe. Immediately following blood sampling, the withdrawn blood volume was replaced with heparinized saline. The blood samples were immediately transferred into EDTA-treated tubes. Blood samples were stored in a 4°C refrigerator until they could be processed, generally within one hour. Plasma was separated from whole blood by centrifugation and stored at –20°C until analysis.

Bioanalytical Procedures

The concentrations in rat plasma were quantified using a validated High Performance Liquid Chromatography (HPLC) system using fluorescent detection. Briefly, preparation of plasma samples involved protein precipitation by organic solvent containing internal standard (7-diethylamino-4-methyl-coumarin), centrifugation, and collection of supernatant. A reverse-phase high performance liquid chromatography (HPLC) method with fluorescence detection (FLD) was used.

Column: Varian, Microsorb-MT C_{18}, 4.6 x 250 mm, 5 µm
Mobile Phase: 23:77 (water:methanol) at 1.2 ml/min
Detection: HP1100 FLD (Ex @ 248 nm and Em @ 476 nm)
Assay Range: Quantitation of 0.025 to 10 µM compound in rat plasma

Data Analysis
HPLC chromatograms were integrated to generate peak areas of test material and internal standard (7-diethylamino-4-methyl-coumarin). Concentrations in the plasma samples were quantified by comparison of the peak area ratios of test article to internal standard in the samples to those in calibration standards prepared in blank rat plasma.
Pharmacokinetic parameters were determined using non-compartmental analysis of plasma concentration-time profiles using WinNonlin pharmacokinetic modeling software Version 3.0 (Pharsight Corporation, Cary, NC). Excel Software (Version 97-SR-2, Microsoft Corp., Redmond, WA) was used for calculation of means, standard deviations, coefficients of variation and students t-tests according to standard methods. The following pharmacokinetic parameters were calculated for each animal. The maximal concentration (C_{max}) and the time required to reach C_{max} (T_{max}) were determined from concentration-time curves after oral administration. The terminal half-life was calculated from the rate constant (k) of the terminal elimination phase, using $t_{1/2} = 0.693/k$, where k was estimated using at least the last 3 quantifiable plasma concentrations, if possible. The area under the concentration-time curve from 0 to the last quantifiable time-point ($AUC_{(0\text{-}t)}$) was calculated using a linear trapezoidal method. AUC from time 0 to infinity was given by; $AUC_{(0\text{-}inf)} = AUC_{(0\text{-}t)} + C_{last}/k$, where C_{last} was the last quantifiable plasma concentration.
Oral bioavailability was calculated as: $(\%F) = \dfrac{AUC_{(0-\inf)}Oral \bullet Dose_{IV}}{AUC_{(0-\inf)}\ IV \bullet Dose_{Oral}}$.

ORDX rat model
The methods for this model are described in reference 1. Serum osteocalcin was measured using a commercially available immunoradiometric assay following the manufacturer's protocol (Immutopics, San Clemente, CA). Serum samples remained frozen until the time of analysis and all samples from an individual experiment were analyzed in a single batch.

OVX Rat Model
Female Sprague-Dawley rats (175-200 g body weight, 3 mo old,) were purchased from Harlan (Indianapolis, IN). Animals were acclimated for 1 wk prior to performing any experimental procedures. The experimental design consisted of ovariectomy (OVX) or sham-OVX performed using the dorsolateral approach as described by Waynforth.[3] OVXed animals were allowed to develop osteopenia with no further experimental manipulations for 7 weeks. At Week 7, animals were anesthetized and scanned by dual-energy x-ray absorptiometry (DXA). Animals were stratified by whole femur BMC and randomly assigned to experimental groups. Treatment with experimental compounds or vehicle began 8 weeks after OVX and continued for 12 weeks. Compound 6a and vehicle were administered daily by oral gavage, whereas estradiol and dihydrotestosterone were administered via daily subcutaneous injection. Body weight was monitored at weekly intervals and the administered doses were adjusted accordingly. All procedures involving animals were approved by Ligand's Institutional Animal Care and Utilization Committee (IACUC).

After 12 weeks of treatment animals were euthanized. The right femur and lumbar spine (L5) were harvested for biomechanical analysis. Biomechanical testing was performed on a QTest2/L materials testing system with a 2 kN load cell (MTS, Eden Prairie, MN). Whole femurs were placed on a custom-designed 3-point bending fixture, consisting of stainless steel pins, 0.63 mm in diameter, with a span of 15

mm between the lower supports. Femurs were tested to failure with a constant crosshead displacement rate of 20 mm/min. The lateral and dorsal spinous processes of lumbar vertebrae L5 were removed with a hand-held grinding/cutting tool (Dremel, Mount Prospect, IL). A low speed saw was used to make two plane-parallel cuts at 4 mm intervals through the vertebral body, effectively removing the growth plates. The resultant cylinder of bone was placed on the loading platen and a compressive load was applied at 20 mm/min in the cranial/caudal direction until failure of the vertebral body. Maximum load, stiffness, and energy absorption were measured from the load-deformation curve for each specimen. Elastic modulus, maximum stress, and toughness were calculated based on standard engineering equations for 3 point bending or compression testing, respectively.

References

[1] van Oeveren, A.; Motamedi, M.; Mani, N. S.; Marschke, K. B.; Lopez, F. J.; Schrader, W. T.; Negro-Vilar, A.; and Zhi, L. Discovery of 6-N,N-bis(2,2,2-trifluoroethyl)amino-4-trifluoromethylquinolin-2(*1H*)-one As a Novel Selective Androgen Receptor Modulator *J. Med. Chem.* **2006**, *49*(21), 6143-6146.

[2] Miner, J.N.; Chang, W; Chapman, M.s; Finn, P.D.; Hong, M.H.; Lopez, F.J.; Marschke, K.B.; Rosen, J.; Schrader, W.; Turner, R.; Van Oeveren, A.; Viveros, H.; Zhi, L.; Negro-Vilar, A. An Orally-Active Selective Androgen Receptor Modulator is Efficacious on Bone, Muscle and Sex Function with Reduced Impact on Prostate *Endocrinology*, **2007**, *148*, 363-373

[3] Waynforth HB, Flecknell PA. Experimental and Surgical Technique in the Rat. London, Academic Press, **1992.**