Discovery of Tertiary Sulfonamides as Potent Liver X Receptor Antagonists

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Contents

General procedure for the preparation of tertiary sulfonamides. 4-Iodobenzylamine (3.20 g, 13.7 mmol), triethylamine (7.65 ml, 104 mmol), and an arylsulfonyl chloride (16.5 mmol, 1.2 equiv) were added to CH_2Cl_2 (40 ml). After allowing this reaction mixture to stir overnight, water was added to precipitate the product, and the solution was acidified with 10 M H_2SO_4. The solids were collected by filtration and triturated with diethyl ether to yield a secondary sulfonamide. In a subsequent step, the secondary sulfonamide (4.1 mmol), a substituted arylboronic acid (4.9 mmol, 1.2 eq), palladium acetate (0.4 mmol), tri(o-tolyl)phosphine (0.8 mmol), and sodium carbonate (8.2 mmol) were added to a mixture of ethylene glycol dimethyl ether (8 ml) and water (2 ml). After heating under a nitrogen atmosphere at 65°C for 4 h, the reaction mixture was then diluted with EtOAc and washed with saturated aqueous $NaHCO_3$. The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Trituration with diethyl ether afforded the biaryl secondary sulfonamide. In a subsequent step, the biaryl sulfonamide (0.09 mmol), a benzylic or heterobenzylic

bromide (0.011 mmol), MP-carbonate (0.045 mmol) and DMF (0.4 ml) were combined and heated at 95°C overnight with stirring. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2, filtered, and concentrated under an N_2 stream. The residue was then dissolved in DMF/MeOH and purified on an Agilent 1100 Series HPLC (70 x 30 mm Phenomenex column packed with Luna 5 micron C18 stationary phase) using 50 to 100% MeCN/water + 0.05% CF_3CO_2H elution to yield the desired biaryl tertiary sulfonamide product.

(**8**) ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.43 (s, 2 H), 4.47 (s, 2 H), 6.87 (td, 1 H), 6.94 (dd, *J*=8.38, 2.50 Hz, 1 H), 7.12 (d, *J*=8.38 Hz, 2 H), 7.31 - 7.47 (m, 7 H), 7.48 - 7.58 (m, 4 H), 7.59 - 7.66 (m, 1 H), 7.83 - 7.89 (m, 2 H); *m/z* 466 $(M+H)^+$.

(**9**) ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.63 (br. s., 2 H), 3.64 (br. s., 2 H), 3.68 (s, 2 H), 7.00 (td, *J*=8.29, 2.54 Hz, 1 H), 7.08 (dd, *J*=8.58, 2.54 Hz, 1 H), 7.22 - 7.26 (m, 1 H), 7.34 (t, *J*=7.61 Hz, 3 H), 7.37 - 7.51 (m, 6 H), 7.57 (dd, *J*=12.88, 7.80 Hz, 4 H), 7.68 (dd, *J*=8.49, 6.54 Hz, 1 H); MS (ESI): *m/z* 416 $(M+H)^+$.

(**10**) ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.52 (br. s., 2 H), 4.76 (br. s., 2 H), 6.96 - 7.10 (m, 1 H), 7.10 - 7.25 (m, 2 H), 7.31 - 7.50 (m, 9 H), 7.53 (br. s., 1 H), 7.56 - 7.71 (m, 5 H); MS (ESI): *m/z* 592 $(M+H)^+$.

(**11**) ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.11 (d, *J*=1.96 Hz, 3 H), 4.44 (s, 2 H), 4.48 (s, 2 H), 6.82 - 6.90 (m, 1 H), 6.90 - 6.99 (m, 1 H), 7.17 (d, *J*=6.60 Hz, 2 H), 7.33 - 7.40 (m, 1 H), 7.40 - 7.48 (m, 2 H), 7.52 - 7.61 (m, 2 H), 7.61 - 7.71 (m, 2 H), 7.80 (d, *J*=7.67 Hz, 1 H), 7.83 - 7.97 (m, 3 H), 8.08 (d, *J*=1.43 Hz, 1 H); MS (ESI): *m/z* 544 $(M+H)^+$

(**12**) ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.30 (s, 3 H), 4.31 (s, 2 H), 4.51 (s, 2 H), 6.88 - 7.03 (m, 1 H), 7.06 - 7.12 (m, 1 H), 7.13 - 7.20 (m, 1 H), 7.22 (d, *J*=8.24 Hz, 2 H), 7.54 (d, *J*=8.24 Hz, 2 H), 7.62 - 7.71 (m, 2 H), 7.71 - 7.78 (m, 2 H), 7.86 - 7.91 (m, 3 H), 7.94 (d, *J*=7.90 Hz, 1 H), 8.02 - 8.09 (m, 1 H); MS (ESI): *m/z* 544 $(M+H)^+$.

(**13**) MS (ESI): *m/z* 508 $(M+H)^+$.

(**14**) MS (ESI): *m/z* 558 $(M+H)^+$ ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.69 (s, 3 H), 3.11 (s, 3 H), 4.60 (br. s., 4 H), 6.76 (t, *J*=8.78 Hz, 1 H), 6.91 - 6.98 (m, 1 H), 6.99 - 7.11 (m, 1 H), 7.15 - 7.31 (m, 2 H), 7.34 (d, *J*=8.00 Hz, 2 H), 7.38 - 7.45 (m, 1 H), 7.47 (d, *J*=8.00 Hz, 2 H), 7.60 - 7.69 (m, 1 H), 7.83 (d, *J*=7.80 Hz, 1 H), 7.90 (dd, *J*=12.98, 7.90 Hz, 2 H), 8.11 (s, 1 H); MS (ESI): *m/z* 558 $(M+H)^+$.

(**15**) ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.29 (s, 3 H), 2.68 (s, 6 H), 3.11 (s, 3 H), 4.51 (s, 2 H), 4.53 (s, 2 H), 6.76 (t, *J*=8.79 Hz, 1 H), 6.92 (s, 2 H), 6.96 - 7.02 (m, 1 H), 7.07 (td, *J*=8.18, 5.86 Hz, 1 H), 7.23 - 7.31 (m, 2 H), 7.44 (d, *J*=8.30 Hz, 2 H), 7.60 - 7.70 (m, 1 H), 7.81 (d, *J*=7.81 Hz, 1 H), 7.91 (d, *J*=7.81 Hz, 1 H), 8.06 - 8.14 (m, 1 H) MS (ESI): *m/z* 586 $(M+H)^+$.

(**16**) ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.28 (s, 3 H), 2.58 (s, 6 H), 3.30 (s, 3 H), 4.41 (s, 2 H), 4.43 (s, 2 H), 7.08 (s, 2 H), 7.11 - 7.21 (m, 3 H), 7.28 (dd, *J*=8.74, 6.24 Hz, 1 H), 7.35 (dd, *J*=8.74, 2.50 Hz, 1 H), 7.66 (d, *J*=8.20 Hz, 2 H), 7.70 - 7.77 (m, 1 H), 7.87 - 7.93 (m, 1 H), 8.00 (d, *J*=7.85 Hz, 1 H), 8.12 (t, *J*=1.69 Hz, 1 H); MS (ESI): *m/z* 586 $(M+H)^+$.

(**17**) ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.27 (s, 3 H), 2.55 (s, 6 H), 3.29 (s, 3 H), 4.34 (s, 2 H), 4.41 (s, 2 H), 6.39 (d, *J*=3.48 Hz, 1 H), 7.03 (d, *J*=2.09 Hz, 1 H), 7.07 (s, 2 H), 7.29 (d, *J*=8.00 Hz, 2 H), 7.61 - 7.82 (m, 3 H), 7.91 (d, *J*=8.00 Hz, 1 H), 8.01 (d, *J*=8.00 Hz, 1 H), 8.13 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.1, 22.8, 41.2, 44.6, 50.0, 110.2, 112.4 (J_{CF} = 2.8 Hz), 117.5, 120.2, 125.8, 126.1, 127.5, 129.5, 129.9, 132.1, 132.2, 132.6, 135.6, 138.7, 140.4, 141.3, 142.1, 143.1, 152.8; ^{19}F NMR ($CDCl_3$) δ -64.7; MS (ESI): *m/z* 592 $(M+H)^+$; HRMS for $C_{29}H_{29}F_3NO_5S_2$ calcd 592.1426; observed 592.1439.

(**18**) ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.10 (d, *J*=2.14 Hz, 3 H), 3.79 (d, *J*=1.78 Hz, 3 H), 4.49 (s, 2 H), 4.70 (s, 2 H), 6.72 (t, 1 H), 6.90 - 6.95 (m, 1 H), 6.95 - 7.05 (m, 1 H), 7.24 - 7.30 (m, 2 H), 7.29 - 7.38 (m, 2 H), 7.48 (s, 1 H), 7.56 - 7.66 (m, 2 H), 7.76 (d, *J*=7.67 Hz, 1 H), 7.88 (d, *J*=7.67 Hz, 1 H), 8.03 (d, *J*=1.61 Hz, 1 H); MS (ESI): *m/z* 548 $(M+H)^+$.

LXR LEADseeker binding assay. Human biotinylated LXRα and LXRβ ligand-binding domains[1] were incubated at 50 and 25 nM, respectively, with 0.25 mg/mL streptavidin-coupled LEADseeker imaging beads (GE Healthcare) in assay buffer (50 mM MOPS (pH 7.5), 50 mM NaF, 0.05 mM CHAPS, 0.1 mg/mL FAF-BSA) for 60 min at 25°C. After being incubated, the receptor-bead slurry was pelleted by centrifugation at 1200g. The supernatant was discarded, and the beads were resuspended in the original volume of assay buffer that contained freshly added 10 mM DTT with gentle mixing. D-Biotin (Pierce Chemical) was added to the resuspended receptor bead solution at 200 μM, and the mixture was allowed to incubate at room temperature for 60 min. After the incubation, [N-methyl-^{3}H]-T1317[2] was added to the receptor/bead mix, and the mixture was mixed gently. The receptor/bead/radioligand mixture (25 μL) was added to each well of an assay plate that contained 0.5 μL of the test compound. The final test compound concentrations were between 300 pM and 20 μM. Plates were incubated at room temperature for 3 h and were then imaged on a ViewLux 1430 ultraHTS microplate imager (Perkin-Elmer).

Demonstration of LXR Antagonism. Antagonist profiles were evaluated in a heterologous reporter assay configured using transient expression of an LXR-Gal4 chimera in CV-1 cells.[3] Gal4 expression constructs using the LBD of each LXR was prepared as described previously.[4] A human SRC-1 construct (representing amino acids 1–1005) was prepared in the pSG5 expression vector (Stratagene Corp., La Jolla, CA).[5] The Gal4 chimera constructs were tested in combination with a reporter plasmid harboring the Gal4 enhancer region linked to the luciferase reporter gene. CV-1 cells were maintained and transiently transfected as described[6] except for inclusion of 7 ng/well of the SRC-1 expression plasmid in the transfections utilizing the Gal4 chimera constructs.

Crystal Structure Determination. A concentrated solution at ~10.5mG/mL of purified human LXRβ ligand binding domain (213-461) with surface entropy reduction mutations[7] (R361S, R362S, C238S, C326S, Q445A, K447A, K448A) containing 5MEq of GSK1305158 and 3MEq of TIF2-740-751 L*XX*LL-III motif peptide KENALLRYLLDK was used to obtain diffraction grade crystals. Vapor diffused hanging drops at 4°C with a 1:1 (v/v) ratio of the protein complex and PEG 2K MME 20%, 0.2mM MgCl2, 0.1mM TRIS HCl pH 8.0 produced ~50µm crystals within 14 days. Prior to flash freezing in liquid N_2, the crystals were transiently mixed with a cryoprotectant solvent consisting of the precipitant solution amended with 20% glycerol and 5% PEG400. X-ray diffraction data at 100°K was collected at the GMCA-CAT, sector 23ID at the Advanced Photon Source synchrotron. HKL2000[8] was used to integrate and scale the $P4_122$ space group data to a 6.5% Rmerge (40.0% high resolution shell) with 96.8% (79.5% high resolution shell) completeness. Phenix AutoMR[9] was used to derive an initial protein model from the protein coordinates of the human LXRβ structure (RCSB access code 1PQ9).[10] The convincing solution contained a single human LXRβ complex in the asymmetric unit that displayed helix 10 mediated homodimer packing with an adjacent molecule. Good quality electron density was observed for both GSK1305158 and the coactivator motif peptide. Multiple cycles of manual model building with COOT[11] and maximum likelihood restrained refinement with hydrogens was performed with Refmac.[12],[13] The final structure was refined to 2.3Å with Rworking / free values of

20.2 / 23.7 and root mean square deviation of 0.011 for the bonds and 1.194 for the angles. The deposited coordinates (RCSB PDB access code 3L0E) have a MolProbity[14] structure quality score of 1.8 (96%). Structure figures were generated with PyMol from Delano Scientific (www.pymol.org) and MOE 2008.10 from the Chemical Computing Group (www.chemcomp.com).[15]

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