Supplementary Materials and Methods:

Cell Culture. COS-1 cells (simian-virus-40-transformed green monkey kidney cells) were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 2 mM glutamime, 10 mM HEPES, 0.15% sodium bicarbonate, 50 units/ml penicillin G, and 50 µg/ml streptomycin and transfected in the same media containing 8% dextran/charcoal treated FBS (HyClone, Logan, UT) in place of the normal FBS.

***Transient Transfection Assays*.** All transfections were performed in a 48-well format. On the morning of day 1, cells were seeded to approximately 60-70% confluency in each well. While the cells were attaching, DNA transfection mixtures were assembled using Fugene6 transfection reagent (Roche Applied Science, Indianapolis, IN). In general, each well was transfected with 25 ng of CMV2-CAR or CMV2-PXR expression plasmid, 25 ng 3.1-RXRα expression plasmid, 100 ng of luciferase reporter, and 10 ng of pRL-CMV (for transfection normalization; Promega, Madison, WI). For all tansfections, the transfection reagent was prepared at a ratio of 1:3 (micrograms of DNA to microliters of transfection reagent) as recommended in the manufacturer's protocol. Within a given experiment, all transfections contained the same total amount of DNA. At the time of transfection (~ 1 h after seeding), cells were approximately 70% confluent. The following day (~18 hr post-transfection), cells were treated with chemical agents, which were all prepared in DMSO. Each treatment was performed in quadruplicate. Because CAR1 is constitutively active, androstanol (10 μM), an inverse-agonist of CAR1 (Forman et al., 1998), is included in the treatment mix to decrease the receptor's constitutive activity, which can then be reversed in the presence of an inverse agonist. The concentrations of other treatments are as follows: kynurenic acid (KYN), 5 and 10 μM; xanthenurenic acid (XAN), 5 and 10 μM, 6-(4-Chlorophenyl)imidazo[2,1-b] [1,3]thiazole-5-carbaldehyde O-3,4-dichlorobenzyl) oxime (CITCO), a positive control for the CAR receptors, 5 μM, and Rifampicin (RFPM), a positive control for PXR, 10 μM. In all treatments, DMSO levels never exceeded 0.2% (v/v). On day 3 (24 h after chemical treatment), cells were washed with PBS and luciferase assays were performed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI) and a Veritas Microplate Luminometer (Turner Biosystems, Sunnyvale, CA). Luciferase assay and stop and glow reagents were diluted with 1x Tris-buffered saline, pH 8.0, to a 0.5x final concentration. All other aspects of the assay were performed in accordance to the manufacturer's protocol. Dilution of luciferase reagent had no effect on normalized luciferase values.

***Statistical Analysis*.** Statistical analyses were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA). A one-way ANOVA with a Dunnett's post hoc test was performed for each nuclear receptor. For CAR1, all treatments were compared to the ANDRO control treatment. For the other receptors, all treatments were compared to the DMSO control values.