Supplemental methods, figures and table

Chemical and reagents

Dimethyl sulfoxide (DMSO), rifampin, 13*cis*RA, *at*RA, cortisol, 6β-hydroxycortisol, 6α-methylprednisolone, dextromethorphan, dextrorphan, dextromethorphan-d_3, 3-methoxymorphinan, 3-hydroxymorphinan, ethyl acetate, and mass spectrometry grade formic acid were purchased from Sigma-Aldrich (St. Louis, MO). Dextorphan-d_3, 13*cis*RA-d_5 and *at*RA-d_5 were obtained from Toronto Research Chemicals (North York, Ontario, Canada). 4-oxo-13*cis*RA and 4-oxo-*at*RA-d_3 and dextrorphan-O-glucuronide were obtained from Santa Cruz Biotechnology (Dallas, TX). Testosterone was purchased from Cerilliant (Round Rock, Texas). GW4064 was obtained from Genentech, Inc. Compound Management Group (South San Francisco, CA). Optima grade LC/MS grade water, acetonitrile, methanol, and formic acid, QuantiGene Plex 2.0 Assay Kits (Panel #13130), Geltrex®, and RNA*later* were purchased from Thermo Fisher Scientific (Waltham, MA). Universal Cryopreservation Recovery Media (UCRM™) was purchased from In Vitro ADMET Laboratories, Inc. (Columbia MD). InvitroGro™ culture media (CP and HI w/o hydrocortisone) and Torpedo™ antibiotic mix were purchased from BioreaclamationIVT (Baltimore, MD). 6β-hydroxytestosterone-d_7 and BD BioCoat™ 96-well Collagen I coated plates were purchased from Corning (Oneonta, NY).

Hepatocyte culture and in vitro assessment of CYP mRNA and activity

For all experiments, hepatocytes were thawed at 37°C, resuspended in pre-warmed UCRM™ Medium, and centrifuged at 100*g* at room temperature for 10 minutes. The supernatants were discarded, and cells were resuspended in InVitroGro CP medium with Torpedo™ to bring up to a concentration of 1.0 x 10^6 cells/mL. Then 65 µL of the cell suspension was added to each well of 96-well, collagen-coated plates. Plates were incubated at 37°C in 5% CO_2 for 4-6 hours to allow cells to adhere to the plate. After the 4-6 hour incubation, the plating media was aspirated and replaced with 0.35 mg/mL Geltrex® in cold HI medium without hydrocortisone containing Torpedo™ as overlaid. The cells were incubated at 37°C in 5% CO_2 for 24 hours. On day two, the cells were dosed with 100 µL/well of incubation media (HI media without hydrocortisone with Torpedo™) containing test material (13*cis*RA, *at*RA, 4-oxo-13*cis*RA, rifampin, or GW4064 depending on the experimental design) or vehicle control (0.1% DMSO). Experiments were conducted out to 48 hours of treatment, and the 48-hour incubation plate was replenished with fresh medium containing Torpedo™ and test materials.

A QuantiGene Plex 2.0 Assay Kit (Panel #13130) from Affymetrix (Santa Clara, CA) was used to quantitate mRNA levels of SHP, CYP2D6, and CYP3A4 using a previously detailed method.[1] To determine CYP activity, each well was washed three times with 100 µL of incubation medium (without hydrocortisone) followed by the addition of 100 µL incubation media containing cocktailed CYP substrates: dextromethorphan (5 µM; CYP2D6 probe substrate) and testosterone (200 µM; CYP3A4/5 probe substrate). The plates were incubated at 37°C in 5% CO_2 for 30 minutes. After a 30 minute incubation, 80 µL of each sample was removed and transferred to wells that contained 160 µL of acetonitrile with internal standards (0.1 µM dextrorphan-d_3 and 1 µM 6β-hydroxytestosterone-d_7). The plates were centrifuged for 10 minutes at 2,000 x *g*, supernatant (60 µL) was removed and added to wells containing 180 µL water. The samples were analyzed by LC-MS/MS as previously described[1] with a slight modification in the MRM transition to quantify the CYP-specific metabolites (dextrorphan *m/z* 258→157, dextrorphan-d_3 261→157 (IS), 6β-hydroxytestosterone *m/z* 305→269, and 6β-hydroxytestosterone-d_7 312→276 (IS)) to determine the activities of CYP2D6 and CYP3A4/5, respectively. The PXR reporter gene assay was performed and analyzed at Puracyp, Inc. (Carlsbad, CA).

Two time-course experiments were conducted to determine the optimal time to characterize changes in expression of SHP, CYP2D6, and CYP3A4 and for assessment of retinoid depletion and interindividual variability in donor response to retinoids. In the first experiment, cells from donor 1 were plated and cultured and then treated with 0.1, 0.3, 1, 3, 10, 30, and 100 μM *at*RA or vehicle control in duplicate for 1, 2, 24, and 48 hours. Levels of SHP, CYP2D6, and CYP3A4 mRNA and activity were assessed at each time point. In the second time-course experiment, cells from the three hepatocyte donors were plated and cultured and then treated with 1 μM of 13*cis*RA, *at*RA, or 4-oxo-13*cis*RA or vehicle control for 48 hours. Aliquots of media were taken at 2, 17, and 24 h after dosing on both treatment days for determination of 13*cis*RA, *at*RA, 4-oxo-13*cis*RA, and 4-oxo-*at*RA concentrations. Retinoid concentrations in media prior to addition to cells were also measured. At the end of the experiment all media was removed and replaced with RNA*later* and cells were stored at -80˚C for analysis of CYP26A1 and RARβ mRNA. Samples were prepared for analysis of retinoid concentrations by precipitating a mixture of 30 μL of sample and 30 μL water with 60 μL acetonitrile containing internal standards (200 nM each of 13*cis*RA-d_5, *at*RA-d_5, or 4-oxo-*at*RA-d_3). Samples were then centrifuged at 18,000 x *g* at 4°C for 30 min and supernantant was taken for analysis by LC-MS/MS as described below for retinoid quantification. The area under the hepatocyte media concentration-time curve from zero to 24 h ($AUC_{0\text{-}24h}$) was calculated using Phoenix WinNonlin v6.3 (Pharsight, St. Louis, MO) using the trapezoidal method and the concentration at time zero was set as the concentration measured in the media prior to addition to cells. The average media concentration (C_{avg}) over each 24-hour treatment for the treated retinoid was determined by dividing the $AUC_{0\text{-}24h}$ by 24 hours. Data are presented as the geometric mean of the C_{avg} determined on the two treatment days.

Determination of retinoid effect on SHP and CYP3A4 mRNA and activity in hepatocytes

To quantify the concentration-effect relationship of 13*cis*RA, *at*RA, or 4-oxo-13*cis*RA on SHP in human hepatocytes, estimates of the maximum increase in expression relative to control (E_{max}) and the retinoid concentration at 50% of the maximum effect (EC_{50}) were determined by fitting the observed concentration-effect data with three-parameter nonlinear regression (equation 1) in GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA). Retinoid concentrations were corrected for retinoid depletion as described in materials and methods. The E_{max} and EC_{50} values are presented as the mean (90% confidence interval) for each donor or replicate experiment. Concentration-dependent increases in CYP3A4 mRNA and activity in hepatocytes treated with 13*cis*RA, *at*RA, and 4-oxo-13*cis*RA were assessed by linear regression and comparing the fitted slope to non-zero.

Protein binding and retinoid free fractions

The plasma and hepatocyte media unbound fraction of retinoids was determined by rapid equilibrium dialysis (RED) and cross-validated using ultracentrifugation. Due to the high protein binding of the retinoids, human plasma and hepatocyte incubation media were diluted (1:10 or 1:20 for plasma and 1:20 for media) in 100 mM potassium phosphate buffer, pH 7.4, then spiked with 1 μM (final concentration) 13*cis*RA, *at*RA, or 4-oxo-13*cis*RA. All experiments were conducted in triplicate. For each retinoid, 200 μL of spiked diluted plasma or diluted hepatocyte incubation media was aliquoted into the matrix chamber of a Thermo Scientific RED Device (Waltham, MA) insert and 400 μL of potassium phosphate buffer was added to the respective buffer chamber. The RED device was incubated at 37°C for 24 hours. An initial time-course experiment was performed to evaluate the time to reach equilibrium, and equilibrium was shown to be established by 24 hours (data not shown). After 24 hours, 75 μL of diluted matrix or buffer sample was precipitated with 75 μL of acetonitrile plus internal standards (200 nM each of

13*cis*RA-d_5, *at*RA-d_5, or 4-oxo-*at*RA-d_3) and centrifuged at 18,000 x *g* at 4°C for 30 min. The supernatant was transferred for quantification of 13*cis*RA, *at*RA, or 4-oxo-13*cis*RA by LC-MS/MS as described below. The fraction unbound in the diluted matrix ($f_{u,dil}$) was calculated as the ratio between the concentration measured in the buffer chamber divided by the concentration measured in the matrix chamber. The fraction unbound (f_u) for each retinoid in undiluted plasma or hepatocyte incubation media was calculated from the data obtained with diluted matrices based on the known dilution factor of the plasma protein concentration and the known relationship between plasma protein concentration ([P]), the binding constant (K_a) and f_u (equation 1):

$$f_u = \frac{1}{1 + K_a[P]} \qquad \text{Equation S1}$$

Equation S1 can be rearranged to yield equation S2,

$$f_u = \frac{1/D}{\left(\left(\frac{1}{f_{u,dil}}\right) - 1\right) + \frac{1}{D}} \qquad \text{Equation S2}$$

in which $f_{u,dil}$ is the unbound faction in diluted matrix, f_u is the unbound fraction in undiluted matrix and D is the dilution factor. The f_u was determined from each $f_{u,dil}$ and D (1:10 or 1:20 for plasma and 1:20 for media). Two experiments were performed with 1:10 dilution in plasma and one experiment each was performed with 1:20 dilution in plasma or hepatocyte media. The final f_u reported for plasma or hepatocyte media is the mean from the separate experiments.

To validate determination of f_u with diluted matrix by RED, binding of retinoids in hepatocyte incubation media was also determined by ultracentrifugation as previously described.[2] 13*cis*RA, *at*RA, or 4-oxo-13*cis*RA were added to hepatocyte incubation media (1 µM final concentration), aliquoted into ultracentrifuge tubes, and either centrifuged in a Sorvall Discovery M150 SE ultracentrifuge (Thermo Fisher Scientific, Waltham, MA) at 435,000 x *g* for 90 minutes at 37°C (n=6 per retinoid) or incubated for 90 minutes at 37°C (n=6 per retinoid). Samples were prepared for LC-MS/MS analysis as described below and the f_u was determined as the ratio of retinoid concentration measured in centrifuged samples divided by the concentration measured in incubated samples. Values of f_u are presented as the mean from the single ultracentrifugation experiment.

Quantification of retinoids

Concentrations of retinoids in hepatocyte media and protein binding experiments were measured using an AB Sciex (Framingham, MA) qTrap 5500 mass spectrometer coupled to an Agilent Technologies (Santa Clara, CA) 1290 Infinity ultrahigh-pressure liquid chromatography system using a previously validated method[3] with some modifications. In brief, analytes were separated using a Kinetex® 100 x 2.1 mM, 1.7 µM C18 column (Phenomenex, Torrance, CA) and a mobile phase of A) water and B) acetonitrile both with 40% methanol and 0.1% formic acid. The gradient was from initial conditions of 50% B for 0.5 min to 95% B for 4 min, then held at 95% B for 2 min before re-equilibration for 1.5 min. The flow rate was 400 µL/min. Analyte detection was performed as previously described with the addition of *m/z* transition of 318→137 for 4-oxo-*at*RA-d_3.[3] The declustering potential, collision energy, and collision exit potential were 62, 18, and 14 for 13*cis*RA and *at*RA, 66, 23, and 16 for 4-oxo-13*cis*RA, 97, 97, and 6 for 13*cis*RA-d_5 and *at*RA-d_5, and 71, 35, and 10 for 4-oxo-13*cis*RA-d_3, respectively. Data were quantified using MultiQuant (AB Sciex, Foster City, CA). The lower limit of quantitation was 5.6 nM in hepatocyte

incubation media and 2.8 nM in plasma for all analytes, and a minimum of six quality control samples were included in each analytical run with %CV < 10 for all analytes.

Quantitation of study drugs and metabolites in serum

To measure 13*cis*RA and its metabolites *at*RA and 4-oxo-13*cis*RA in serum collected from study day 15, serum was diluted 20-fold in water prior to addition of 100 µL of acetonitrile with 13*cis*RA-d_5 (250 nM), *at*RA-d_5 (100 nM), and 4-oxo-*at*RA-d_3 (500 nM) as internal standards. Samples were centrifuged at 16,000 x *g* for 10 min at 4°C, chilled at 4°C for 10 min, and centrifuged again at 16,000 x *g* for 10 min. Analytes were quantified on an Agilent Technologies (Santa Clara, CA) 1290 Infinity ultrahigh-pressure liquid chromatography system coupled to an AB Sciex (Framingham, MA) qTrap 5500 mass spectrometer as previously described.[3] The percentage coefficient of variation (%CV) for all analytes was ≤ 11% and the lower limit of quantification (LLOQ) in serum was 2.5 nM for 13*cis*RA and *at*RA and 50 nM for 4-oxo-13*cis*RA. Quality control samples were included in all analytical runs and had to meet standard assay validation criteria for run acceptance.

To prepare serum and urine samples for analysis of dextromethorphan and metabolites, 100 µL of serum or urine was added to 100 µL of acetonitrile with 10 nM dextromethorphan-d_3 as internal standard, the samples were centrifuged at 16,000 x *g* for 10 min at 4°C, chilled at 4°C for 10 min, and centrifuged again at 16,000 x *g* for 10 min. Supernatant was collected and concentrations of dextromethorphan, dextrorphan, dextrorphan-O-glucuronide, 3-hydroxymorphinan, and 3-methoxymorphinan were measured with an Agilent Technologies (Santa Clara, CA) 1290 Infinity ultrahigh-pressure liquid chromatography system coupled to an AB Sciex (Framingham, MA) qTrap 5500 mass spectrometer as previously described[4] with minor modifications. Briefly, analytes were separated with a Kinetex® 100 x 2.1 mm 2.6 µm EVO C18 column (Phenomenex, Torrance, CA) with a mobile phase of A) water and B) acetonitrile both with 0.1% formic acid and a gradient elution of 10% B for 0.5 min, increased to 90% B for 2 min and held for 1.5 min before returning to initial conditions and held for 2 min. Analyte detection was performed with electrospray ionization operated in positive ion mode, with the exception of dextrorphan-O-glucurdonide which was detected in negative ion mode, with *m/z* transitions 272→128 for dextromethorphan, 258→157 for dextrorphan, 432→256 for dextrorphan-O-glucurdonide, 244→157 for 3-hydroxymorphinan, 258→171 for 3-methoxymorphinan, and 275→128 for dextromethorphan-d_3. Standard curves in serum ranged from 0.3 – 30 nM for dextromethorphan and dextrorphan with quality control sample %CV ≤ 8% and ≤ 17%, respectively. In urine all analytes had LLOQs of less than 7.8 nM except for dextrorphan-O-glucuronide which had a LLOQ of 78.1 nM. The %CV for analytes in urine was ≤ 7% expect for dextrorphan-O-glucuronide that had a %CV of ≤ 17%. Urine samples initially quantified above the standard curve ranges were diluted with blank urine and reanalyzed.

Concentrations of cortisol and 6β-hydroxycortisol were measured in serum and urine, respectively, using liquid-liquid extraction and monitored by LC-MS/MS as described previously.[4,5] Serum samples (250 µL) with an added 750 µL water or urine samples (1 mL) were spiked with 100 nM 6α-methylprednisolone as internal standard and then extracted twice with 3 mL of ethyl acetate. After each extraction, samples were centrifuged and the organic phase was collected. The combined ethyl acetate layers were evaporated under nitrogen and reconstituted in 100 µL 1:1 methanol:water for analysis by LC-MS/MS with an Agilent Technologies (Santa Clara, CA) 1290 Infinity ultrahigh-pressure liquid chromatography system coupled to an AB Sciex (Framingham, MA) qTrap 5500 mass spectrometer. A Thermo Hypersil Gold C18 100 x 2.1mm, 1.9 µm column (Waltham, MA) was used for analyte separation with a mobile phase of A) water plus 0.1% formic acid and B) acetonitrile. A gradient elution of 10% B for 0.5 min, increased to

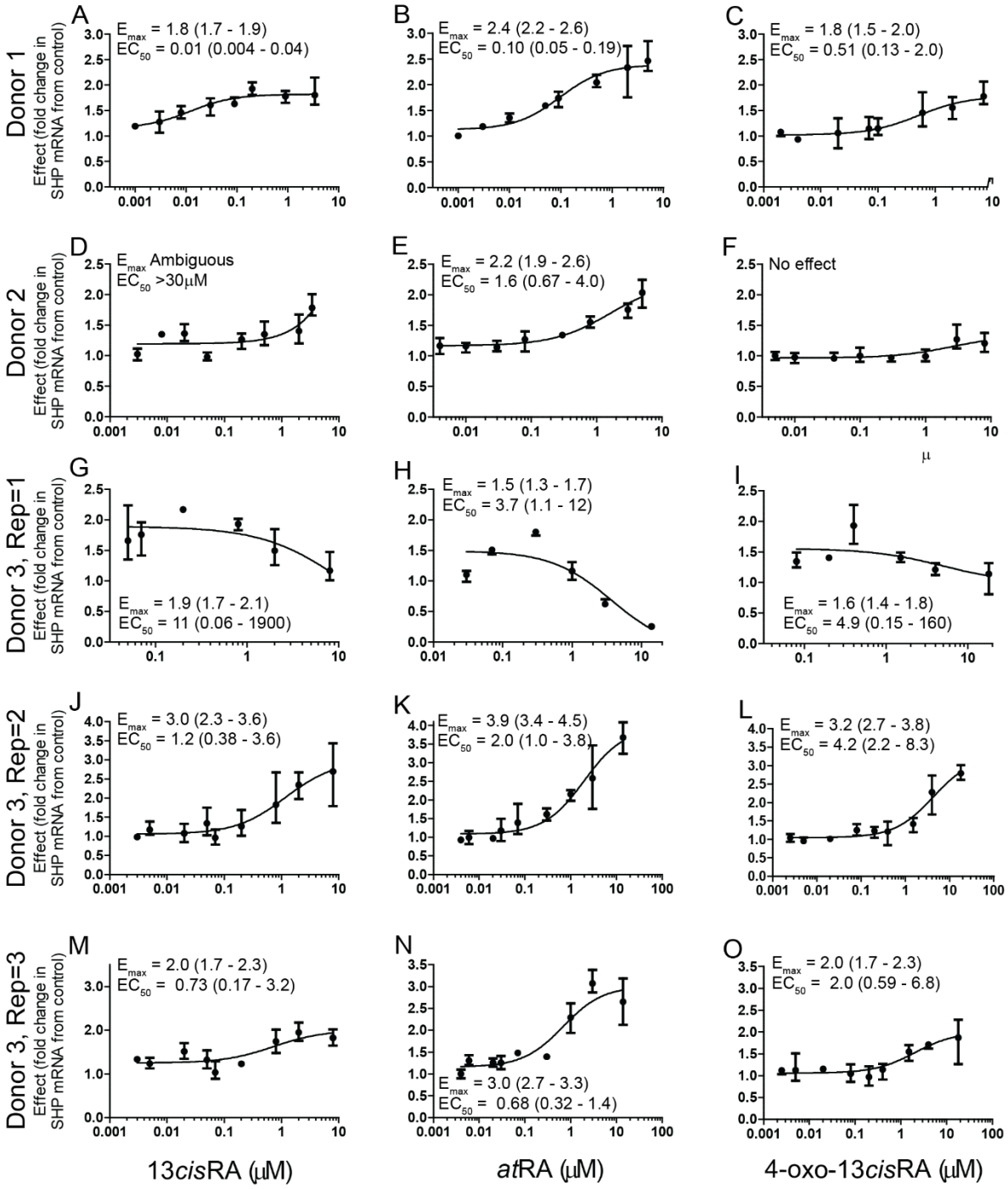
90% B over 3 min and held for 1.5 min before returning to initial conditions and held for 2 min was used. Cortisol detection was performed with electrospray ionization operated in positive ion mode with *m/z* transition of 363→121 and 6β-hydroxycortisol and 6α-methylprednisolone were detected in negative ion mode with *m/z* transitions of 423→347 for 6β-hydroxycortisol and 421→345 for 6α-methylprednisolone. The LLOQ was 3.1 nM for cortisol in serum and 1.6 nM for both cortisol and 6β-hydroxycortisol in urine with CV% of ≤ 15% for all analytes.

Pharmacokinetic analysis

For pharmacokinetic analysis of dextromethorphan and its CYP2D6-generated metabolite dextrorphan, the maximum concentration (C_{max}) was reported from the observed concentration versus time profiles and the area under the serum concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) was determined using the linear up-log down trapezoidal rule. The half-life ($t_{1/2}$) was calculated as ln2 divided by the slope of the terminal linear phase. The oral clearance (CL/F) for dextromethorphan was calculated as dose divided by $AUC_{0-\infty}$. The formation clearance (Cl_f) of dextrorphan was determined by dividing the sum of the molar equivalents of dextrorphan and its metabolite dextrorphan-O-glucuronide recovered in urine over 24 hours following dosing of dextromethorphan by the AUC_{0-24h} of dextromethorphan. The metabolite to parent AUC ratios for dextrorphan to dextromethorphan in serum were calculated from the quotient of the $AUC_{0-\infty}$. The urinary metabolite to parent ratios were determined by recovery of dextrorphan and dextrorphan-O-glucuronide divided by the recovery of dextromethorphan in 24 hours following dextromethorphan dosing. The Cl_f of the CYP3A4-specific metabolite of dextromethorphan, 3-methoxymorphinan, was determined by dividing the molar equivalents of 3-methoxymorphinan and its metabolite 3-hydroxymorphinan recovered in urine over 24 hours following dosing of dextromethorphan by the AUC_{0-24h} of dextromethorphan. The Cl_f of 6β-hydroxycortisol was determined by dividing the molar amount of 6β-hydroxycortisol recovered in urine over 8 hours following dosing of dextromethorphan by the AUC_{0-8h} of cortisol as previously described.[5] The average steady state concentrations (C_{ss}) for 13*cis*RA, 4-oxo-13*cis*RA, and *at*RA following two weeks of 13*cis*RA dosing were determined by dividing the AUC_{0-12h} calculated for each analyte, respectively, by 12 h.

Real time PCR for mRNA measurements in human hepatocytes and in mouse liver

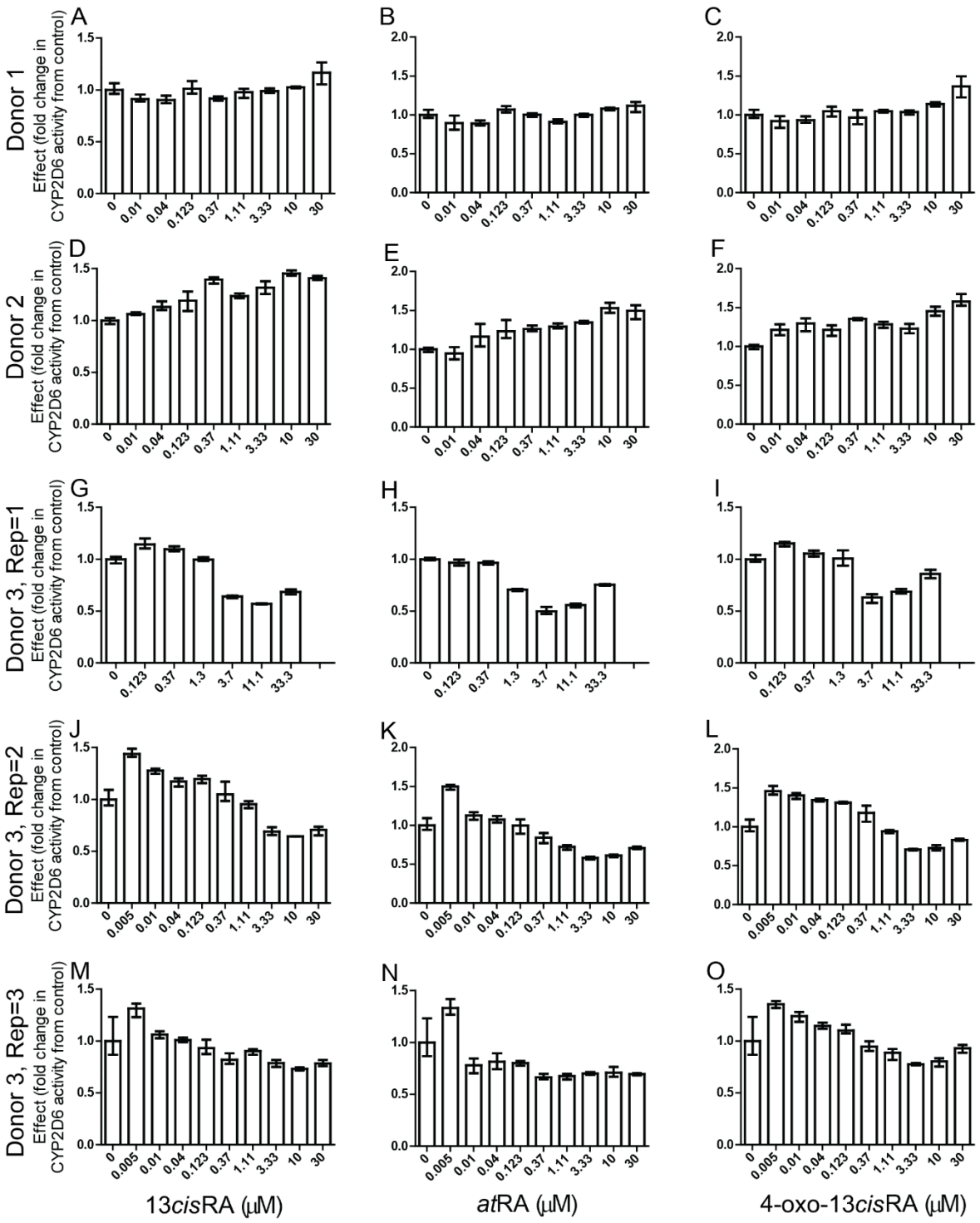
Expression of CYP26A1 and RARβ mRNA in human hepatocytes and expression of Shp, Cyp7a1, Cyp8b1, Cyp2d9, Cyp2d10, Cyp2d11, Cyp2d22, Cyp2d40, Cyp26a1, and Gapdh mRNAs in mouse liver were quantified as previously described using q-rt-PCR using a StepOnePlus (Applied Biosystems) instrument with TaqMan real-time gene expression master mix.[6] Hepatocyte mRNA was extracted from cells in RNA*later* using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. To extract mRNA from mouse liver, approximately 100 mg of mouse liver was homogenized with 1mL of TRI reagent (Invitrogen, Grand Island, NY) and total mRNA was extracted with TRIzol (Invitrogen, Carlsbad, CA). Total mRNA extracted from hepatocytes or mouse liver was quantified on a Nanodrop 2000c Spectrophotometer (Thermo Fischer Scientific, Waltham, MA). cDNA was generated using 1 µg RNA and TaqMan Reverse Transcription Reagents (catalog number N8080243, Applied Biosystems, Carlsbad, CA). The primer probe pairs and TaqMan gene expression assays were obtained from Applied Biosystems and included Hs00175627_m1 (human CYP26A1), Hs00977140_m1 (human RARβ), Hs99999905_m1 (human GAPDH), Mm0044278_m1 (mouse Shp, Nrob2), Mm00484150_m1 (mouse Cyp7a1), Mm00501637_s1 (mouse Cyp8b1), Mm00651731_m1 (mouse Cyp2d9), Mm00731648_m1 (mouse Cyp2d10), Mm04205381_gH (mouse Cyp2d11), Mm00530542_m1 (mouse Cyp2d22), Mm01303815_m1 (mouse Cyp2d40), Mm00514486_m1 (mouse Cyp26a1), and Mm99999915_g1 (mouse Gapdh). GAPDH was used as the housekeeping gene. All human hepatocyte samples were analyzed in triplicate, and data presented are the mean of the triplicate analyses. All mouse liver samples were analyzed in duplicate. Changes in target mRNA were measured using relative quantification (fold-difference) and the $\Delta\Delta C_T$ method. For purposes of estimating a maximum possible induction of CYP26A1 in human hepatocytes, any undetermined cycle threshold (C_T) values were assigned a C_T value of 39.



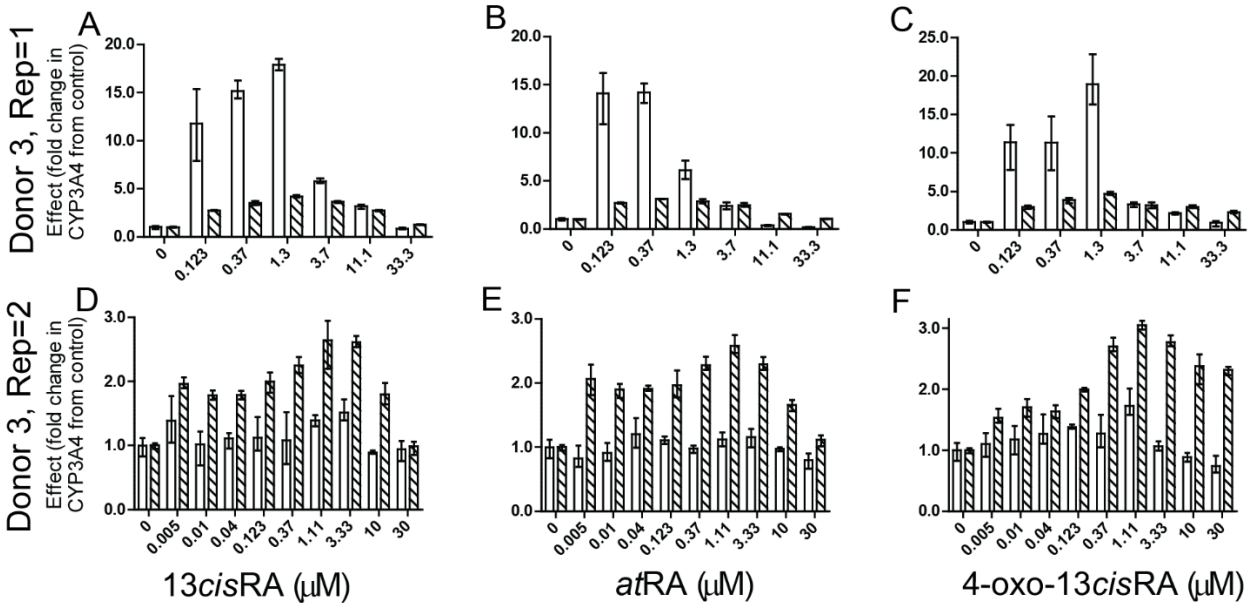
Supplemental Figure 1. The effects of treatment with 13*cis*RA, *at*RA, and 4-oxo-13*cis*RA on SHP mRNA in donor 1 (A, B, C), donor 2 (D, E, F), and in three replicate experiments in donor 3 (G - O) hepatocytes. The points show the mean and range of the measured effect in comparison to control and the line shows the fit of the data. The x-axis shows actual retinoid concentration corrected for depletion. The E_{max} and EC_{50} (μM) shown were fit as described in the methods and are presented as mean (90% CI). The control GW4064 (1μM) increased SHP mRNA by an average 2.4-, 1.2-, and 2.7-fold in donors 1, 2, and 3, respectively.

Supplemental Table 1. The EC_{50} and E_{min} values for CYP2D6 mRNA downregulation for the three individual experiments shown in Figure 1 panels g, h and i.

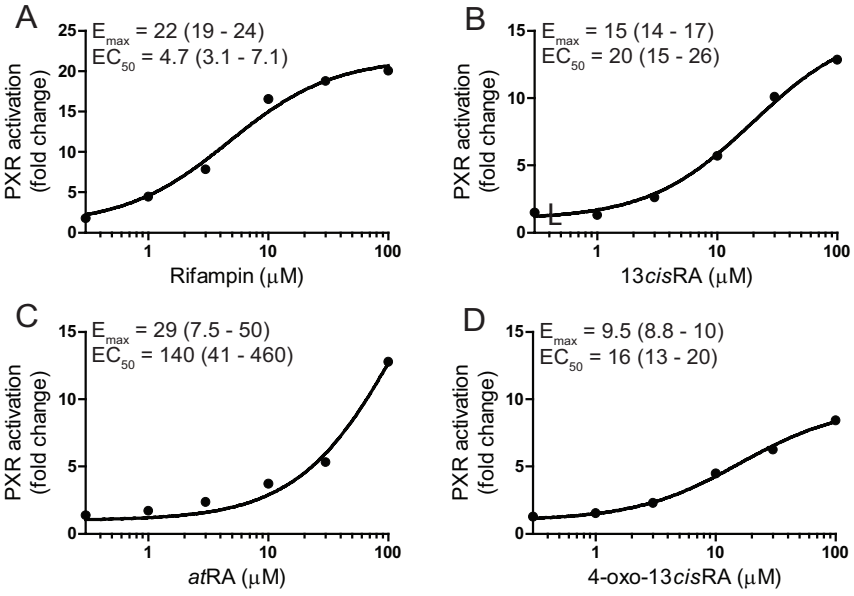
Replicate	Endpoint	13*cis*RA	*at*RA	4-oxo-13*cis*RA
1	E_{min}	0 (0 – 0.05)	0 (0 – 0.03)	0 (0 – 0.06)
	EC_{50}	0.30 (0.18 – 0.50)	0.20 (0.14 – 0.28)	0.55 (0.28 – 1.1)
2	E_{min}	0.05 (0 – 0.11)	0.07 (0.03 – 0.11)	0.05 (0 – 0.11)
	EC_{50}	0.08 (0.05 – 0.14)	0.05 (0.03 – 0.08)	0.14 (0.09 – 0.22)
3	E_{min}	0.19 (0.11 – 0.27)	0.15 (0.13 – 0.18)	0.19 (0.10 – 0.27)
	EC_{50}	0.04 (0.02 – 0.07)	0.006 (0.004 – 0.008)	0.07 (0.04 – 0.12)



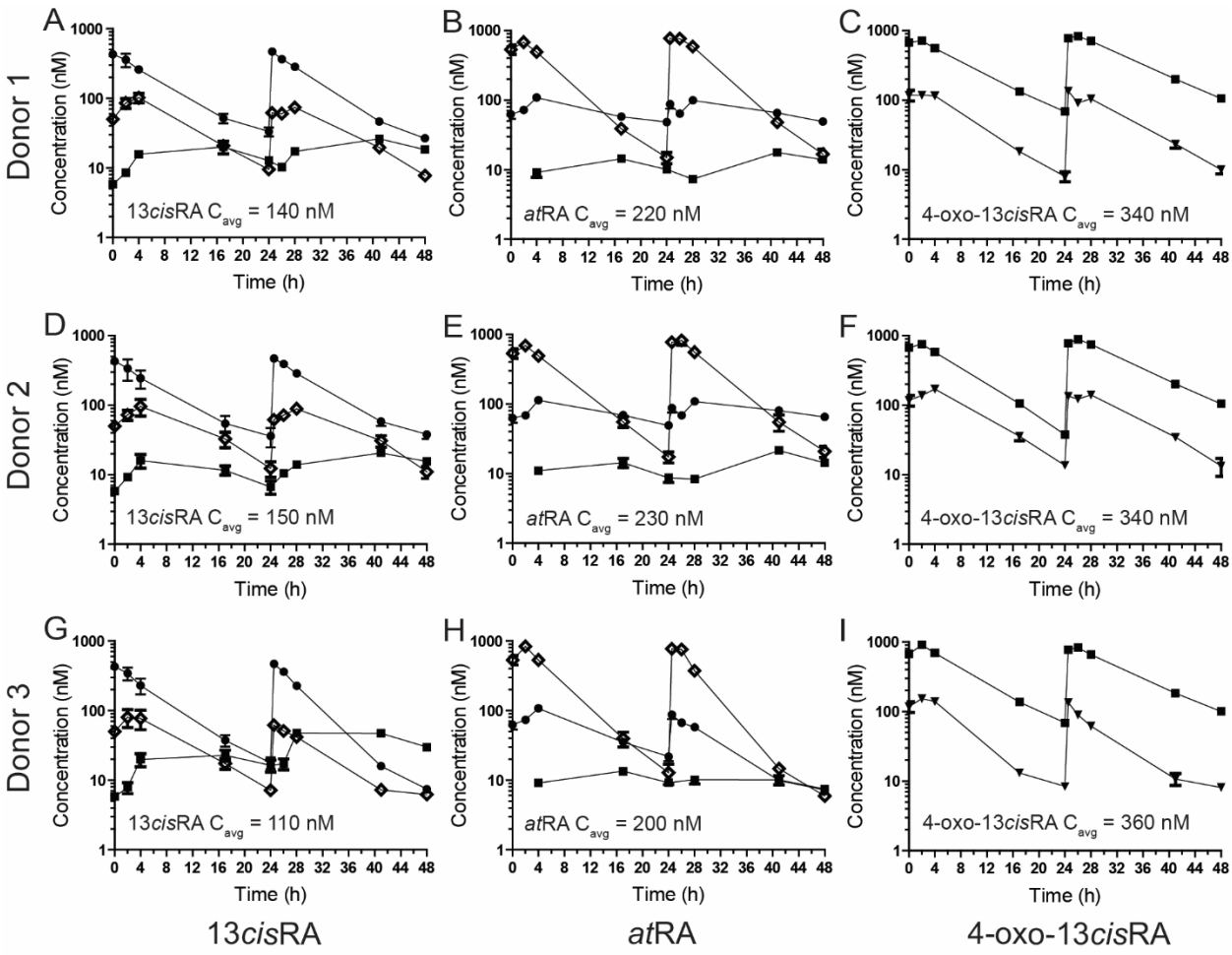
Supplemental Figure 2. The effects of 13*cis*RA, *at*RA, and 4-oxo-13*cis*RA on CYP2D6 activity in donor 1 (A, B, C), donor 2 (D, E, F), and in three replicate experiments in donor 3 (G - O) hepatocytes. Data are presented as mean and range of the measured effect (formation of dextrorphan from dextromethorphan in a 90 min incubation following 48 h retinoid treatments). The SHP inducer control, GW4064 (1μM), increased CYP2D6 activity by an average 1.4-, 1.8-, and 1.3-fold in donors 1, 2, and 3, respectively.



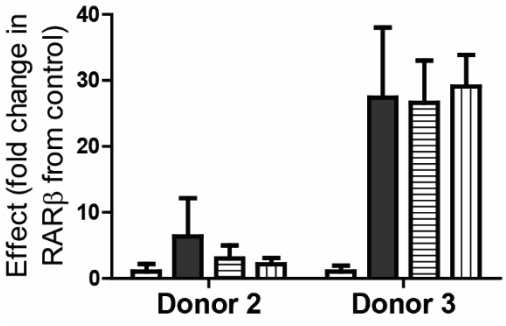
Supplemental Figure 3. The effects of 13*cis*RA, *at*RA, and 4-oxo-13*cis*RA on CYP3A4 mRNA (white bars) and activity (hashed bars) in two replicate experiments in donor 3. Data are presented as mean and range of the measured effect (n=3 replicates per experiment). The control rifampicin (25μM) increased CYP3A4 mRNA 85- and 125-fold and CYP3A4 activity 30- and 10-fold in experiments 1 and 2, respectively.



Supplemental Figure 4. Activation of PXR by 13*cis*RA, *at*RA, and 4-oxo-13*cis*RA in PXR reporter assay. In the same assay rifampicin control (10μM) resulted in a 19-fold activation of PXR signaling



Supplemental Figure 5. Concentrations vs time curves of the retinoids in human hepatocytes following treatment with 1 µM 13*cis*RA, *at*RA, or 4-oxo-13*cis*RA. Panels A, B and C show donor 1, panels D, E and F show donor 2 and Panels G, H and I show donor 3. The treatments shown are 13*cis*RA in panels A, D and G, *at*RA in panels B, E and H and 4-oxo-13*cis*RA in panels C, F and I. The experiments were conducted as described in the methods section. Concentrations of the treated retinoid and metabolites are presented as mean and standard deviation (n=3 replicates per donor) with 13*cis*RA shown as circles, *at*RA as diamonds, 4-oxo-13*cis*RA as squares and 4-oxo-*at*RA as inverted triangles. The geometric mean C_{avg} of the respective retinoids in each is shown in the panels.



Supplemental Figure 6. Change in expression of RARβ mRNA in human hepatocytes following treatment with vehicle control (open bars), 13*cis*RA (black bars), *at*RA (horizontal striped bars) or 4-oxo-13*cis*RA (vertical striped bars). Data are presented as mean and standard deviation (n=3 replicates per experiment).

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