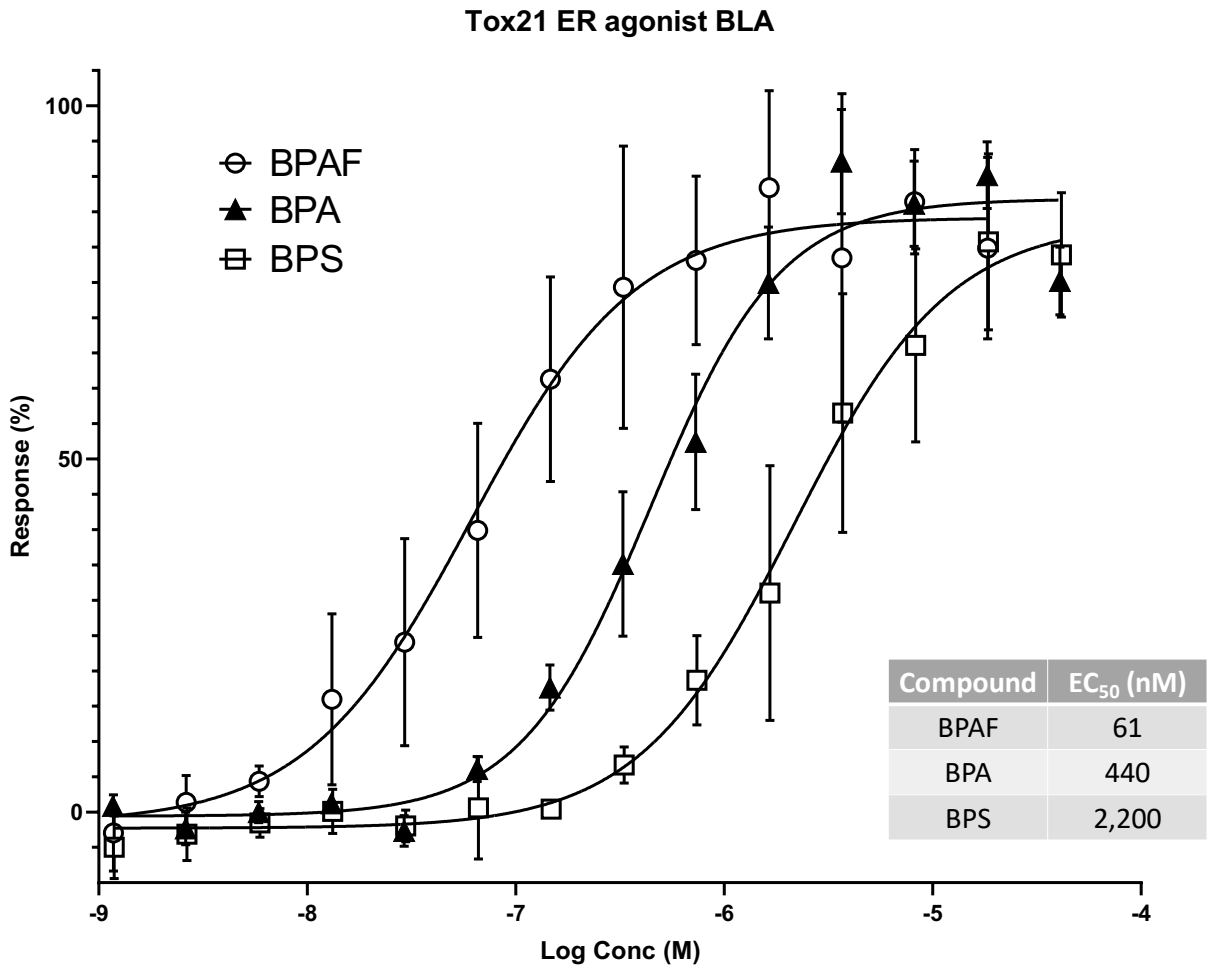
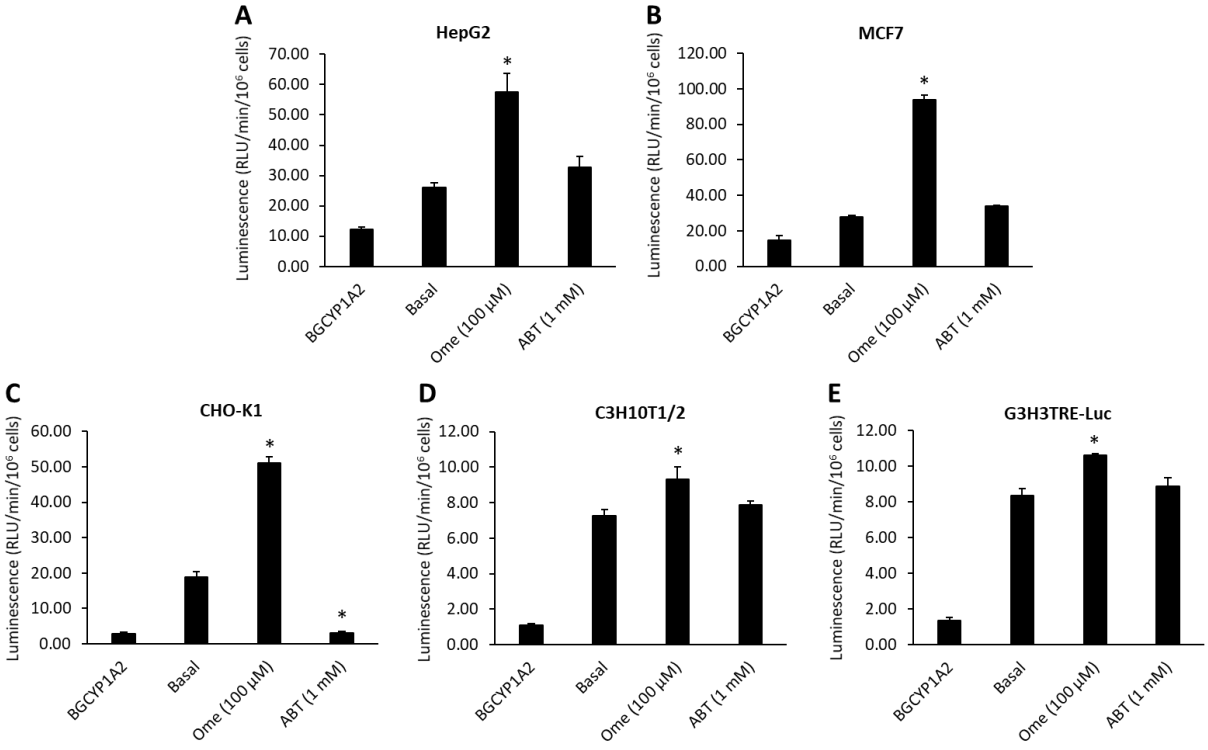


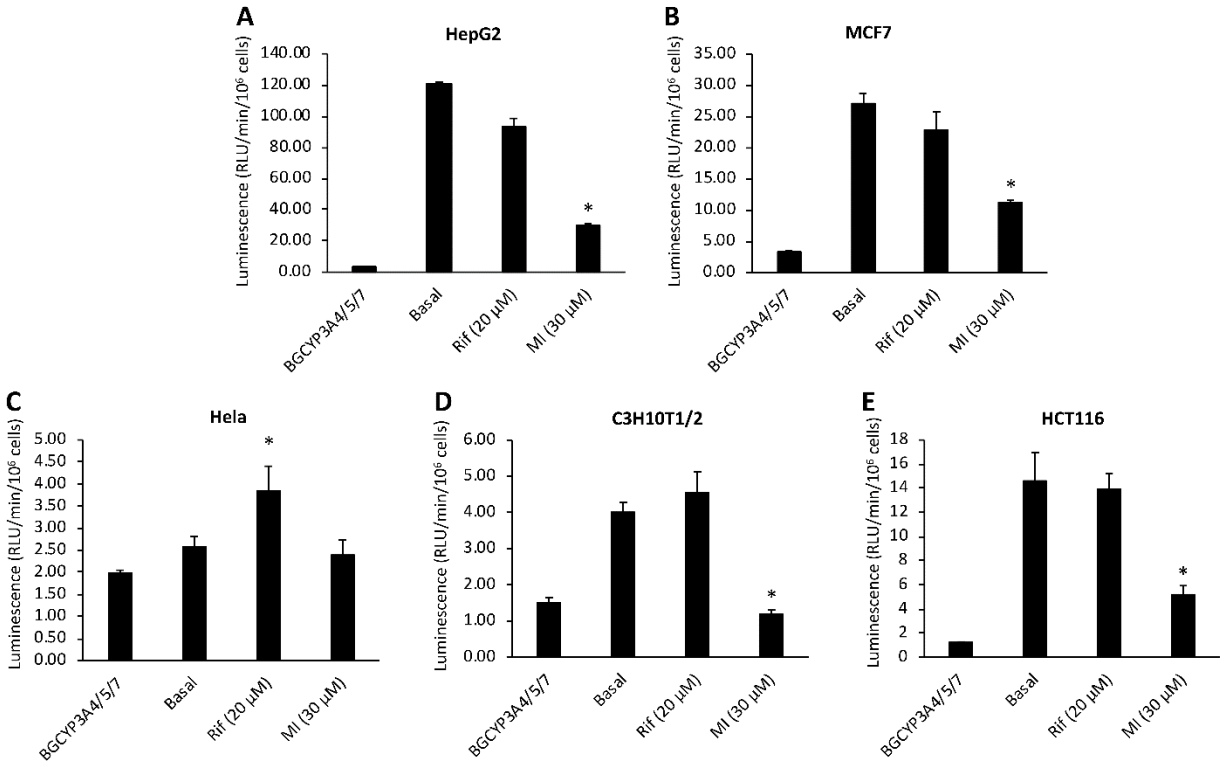
Supplemental Figure 1. Tox21 ER agonist bioactivity screening data for BPAF, BPS, & BPA.

Tox21 estrogen receptor agonist assays (i.e., tox21-er-luc-bg1-4e2-agonist) curve overlays for representative experimental runs with BPAF, BPS, and BPA demonstrating the relative potency differences of estrogen receptor agonist activity with these structural analogues. Data represent normalized responses of 3 independent experimental runs in the form of mean ± SD. Curve overlay comparing Tox21 estrogen receptor agonist activities for bisphenol A, bisphenol AF, and bisphenol S concentration as archived in https://tripod.nih.gov/tox21, and published in Sci Rep. 2014; 4: 5664.



Supplemental Figure 2. P450 Glo CYP1A2 induction and inhibition in Tox21 cell lines.

Tox21 cell lines were cultured as a monolayer in a 96-well plate at ~50,000 cells/well for 24 hours. Cells were subsequently pretreated with the vehicle 0.1% DMSO (Basal), or OMP (100 µM), a prototype CYP1A2 inducer, or ABT (1 mM), a pan-P450 inhibitor, for 24 hours. Assay background (BGCYP1A2) was measured in medium without cells. After incubations, culture media were replaced with fresh medium containing Luciferin-1A2 P450-Glo substrate (6 µM), and cells were incubated for an additional 60 minutes. CYP450-Glo bioluminescent assays were performed. HepG2 cells (A). MCF7 cells (B). CHO-K1 cells (C). C3H10T1/2 cells (D). G3H3TRE-Luc cells (E). Data are presented as the mean ± SD (n = 4). * $p < 0.05$ compared with basal.



Supplemental Figure 3. P450 Glo CYP3A4 induction and inhibition in Tox21 cell lines.

Tox21 cell lines were cultured as a monolayer in a 96-well plate at ~50,000 cells/well for 24 hours. Then, cells were pretreated with the vehicle 0.1% DMSO (Basal), or RIF (20 µM), well known CYP3A4 inducer, or MI, (30 µM), a CYP3A4 inhibitor, for 24 hours. Assay background (BGCYP3A4) was measured in medium without cells. After treatments the medium was replaced with fresh medium containing Luciferin-3A4 P450-Glo substrate (3 µM), and cells were incubated for an additional 60 minutes. CYP450-Glo bioluminescent assays were performed. HepG2 cells (A). MCF7 (B). Hela cells (C). C3H10T1/2 cells (D). HCT116 cells(E). Data are presented as the mean ± SD (n = 4). * $p < 0.05$ compared with basal.

Supplementary Table 1. Analytes and Stable Label Internal Standards

Analyte or ISTD	Manufacturer	Catalog #	Lot #
Acetaminophen	Sigma, St. Louis, MO	A7085	053K0040
$^{13}C_2$-^{15}N-Acetaminophen	Cambridge Isotope Laboratories, Tewksbury, MA	CNLM-3726-1.2	SDED-003
Hydroxybupropion	BD Gentest, Franklin Lakes, NJ	451711	00081
Hydroxybupropion-d_6	Cerilliant, Round Rock, TX	H-062	FN01051503
1`-Hydroxymidazolam	BD Gentest	451038	51142
1`-Hydroxymidazolam-d_4	Cerilliant	61-546	FN092807-02

Supplementary Table 2. LC/MS/MS Method Instrumentation and Analysis Parameters

Instrumentation

Mass Spectrometer	API 5000 Triple Quadrupole Mass Spectrometer with Turboion Spray source (SCIEX, Framingham, MA)
HPLC	Waters Acquity UPLC with autosampler and column compartment (Waters, Milford, MA)

Chromatography Conditions

Column	Phenomenex Luna C18 (50 x 2 mm, 5 μm) with C18 guard cartridge (Phenomenex, Torrance, CA)
Injection Volume	10 microliters
Mobile Phase	A: 0.05% Formic Acid in 5 mM Ammonium Formate, B: 0.05% Formic Acid in Acetonitrile:Methanol (95:5)
Flow Rate	0.7 mL/min
Gradient	Initially 2% B, change linearly to 5% B over 0.5 min. Change linearly to 71% B over 2 min, then return to initial conditions over 0.5 min. Run time 5 min.

Supplementary Table 3. MS Parameters: Positive TurboSpray Ionization

Parameter	Acetaminophen	Hydroxybupropion	1-Hydroxymidazolam
Polarity	Positive	Positive	Positive
Ion Source	TurboSpray	TurboSpray	TurboSpray
Resolution	Unit	Unit	Unit
Curtain Gas	10	10	10
Gas 1	50	50	50
Gas 2	50	50	50
IonSpray Voltage	2500.00	2500.00	2500.00
Source Temperature	650	650	650
Collision Gas (CAD)	8	8	8
Entrance Potential	10.00	10.00	10.00
Declustering Potential	41	46	76
Collision Cell Exit Potential	18	16	31
Collision Energy	23	17	24
Analyte MRM	151.95 → 110.0	256.12 → 238.0	342.07 → 324.0
Internal Standard MRM	154.95 → 111.0	262.10 → 243.8	346.00 → 328.0

Supplementary Table 4. BPAF Phase II Metabolites Inclusion List

Metabolite	Mass (m/z)
BPAF-S	415.05122
BPAF-2S	494.96485
BPAF-G	511.08331
BPAF-2G	687.11540
BPAF-GS	591.04012

Supplementary Table 5. BPS Phase II Metabolites Inclusion List

Metabolite	Mass (m/z)
BPS-S	328.97952
BPS-2S	408.93633
BPS-G	425.05479
BPS-2G	601.08688
BPS-GS	505.01161

Supplementary Table 6. MS Parameters for 7-HC sulfate

.

Parameter	MS Setting 7-hydroxycoumarin sulfate
Polarity	Negative
Ion Source	HESI
Resolution	70,000
Sheath Gas Flow Rate	48
Aux Gas Flow Rate	11
Sweep Gas Flow Rate	2
Spray Voltage (kV)	3.40
Capillary Temperature (°C)	300
S-lens RF Level	40.0
Aux Gas Heater Temp (°C)	413

Supplementary Table 7. MS Parameters for 7-HC glucuronide

Parameter	MS Setting 7-hydroxycoumarin glucuronide
Polarity	Positive
Ion Source	HESI
Resolution	70,000
Sheath Gas Flow Rate	48
Aux Gas Flow Rate	11
Sweep Gas Flow Rate	2
Spray Voltage (kV)	3.00
Capillary Temperature (°C)	300
S-lens RF Level	60
Aux Gas Heater Temp (°C)	413

Supplementary Table 8. MS Parameters for BPAF and BPS Metabolites

Parameter	MS Setting BPAF or BPS Metabolites
Polarity	Negative
Ion Source	HESI
Resolution	70,000
Sheath Gas Flow Rate	48
Aux Gas Flow Rate	11
Sweep Gas Flow Rate	2
Spray Voltage (kV)	2.20
Capillary Temperature (°C)	300
S-lens RF Level	50
Aux Gas Heater Temp (°C)	413

Supplementary Table 9. Metabolic clearance assay MS Parameters (7-HC, BPAF, and BPS)

Parameter	MS Setting 7-hydroxycoumarin/BPAF/BPS
Polarity	Negative
Ion Source	HESI
Resolution	70,000
Sheath Gas Flow Rate	48
Aux Gas Flow Rate	11
Sweep Gas Flow Rate	2
Spray Voltage (kV)	3.40
Capillary Temperature (°C)	300
S-lens RF Level	40.0
Aux Gas Heater Temp (°C)	413