Supplemental Methods Materials:

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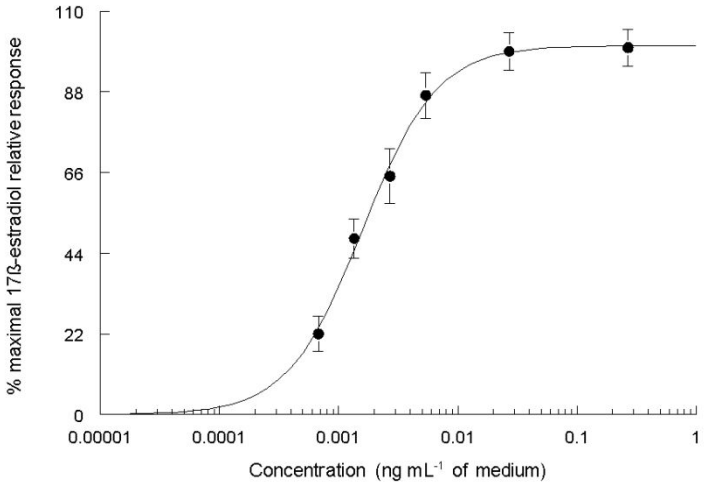
SUPPLEMENTAL FIGURE

Supplemental Figure 1. Dose response standard curve. MMV-Luc cell line treated with increasing concentrations of 17beta-estradiol (n=7). Luciferase activity measured after 24h



SUPPLEMENTAL TABLES

Supplemental Table 1. *EC_{50} values for each Reporter Gene Assay and standard hormone used.*

Reporter Gene Assay	**Hormone**	**EC_{50} (ng/mL)**
Estrogen	17beta-estradiol	0.01
Androgen	Testosterone	0.16
Progesterone	Progesterone	0.6
Glucocorticoid	Hydrocortisone	53

Supplemental Table 2*. Analyte spiking concentrations and % recovery rate for the Bond Elut extraction method.*

RGA	**% recovery**	**Hormone and spiking concentration used**
Androgen	75 – 80	17beta-estradiol – 0.5 ng/mL (2nM)
Estrogen	75 – 80	Testosterone – 2.9 ng/mL (10nM)
Progesterone	~ 60	Progesterone – 5 ng/mL (16nM)
Glucocorticoid	~100	Hydrocortisone – 100 ng/mL (276 nM)

Extraction of hair products

One gram of each product was weighed in a glass tube to which ethyl acetate was added at a ratio of 5ml to 1g product. Each sample was (un)spiked with 17beta-estradiol, testosterone, progesterone, and hydrocortisol and thenvortexed and sonicated for 5-15 minutes. After sonication, samples were diluted with n-hexane to a 25 mL volume. Bond Elut columns were used for extraction and were pre-conditioned with 3 mL of methanol, 3 mL n-hexane, and 3mL ethyl-acetate. The sample was then added into the column and followed by washing with 4 mL of n-hexane: ethyl acetate (4:1, v:v) and eluted by 6 mL of methanol. Eluent was evaporated under a nitrogen stream at 40°C and re-suspended in 250 µL methanol.

Thiazolyl blue tetrazolium bromide (MTT) analysis

Cells were seeded as for the RGAs in clear, flat-bottomed 96-well plates and incubated for 24 hours. The extracted test compounds at a final MeOH concentration of 0.5% and solvent control (0.5% v:v MeOH in media) were added to the cells and incubated for 48 hours. The supernatant was discarded and cells washed once with 200 µL PBS. Then, 50 µL of thiazolyl blue tetrazolium bromide (MTT) solution (2 mg/mL stock in PBS, diluted 1:6 in media) was added to each well and incubated for 3 hours at 37 °C. In this assay, viable cells convert the soluble yellow MTT into insoluble purple formazan by the action of mitochondrial succinate dehydrogenase. The supernatant was removed and 200 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The plate was incubated at 37 °C for 10 minutes with agitation. Optical density (OD) was measured using a Sunrise spectrophotometer (TECAN, Switzerland) at 570 nm with a reference filter at 630 nm. All samples were assessed in triplicate wells and in three independent exposures. Viability was calculated as the percentage absorbance of the sample when compared with the absorbance of the solvent control.

Reporter Gene Assay cell lines

Cells were seeded at a concentration of 4×10^5 cells/mL in 100 µL media into white walled 96 well plates with clear flat bottoms (Greiner Bio-One, Germany). The cells were incubated for 24h prior to being exposed to the samples. The samples were diluted in assay media to a final 1/500 dilution from the original extract while maintaining MeOH concentration below 0.5%. The cells were exposed to samples for 24h (MMV-Luc cell line) or 48h (for all other cell lines). The supernatant was discarded and the cells washed twice with phosphate buffered saline (PBS) prior to lysis with 20 µL cell culture lysis buffer (Promega, Southampton, UK). Finally, 100 µL luciferase (Promega, Southampton, UK) was injected into each well and luciferase activity measured using the Mithras Multimode Reader (Berthold, Other, Germany). All experiments were performed in triplicate. To determine activity, the response of the cell lines to the product samples was measured as relative luciferase response units and compared to the extracted solvent control (0.5% methanol in media) which was used as a negative control for sample analysis.

For the agonist test, the standard curves used for each cell line were: 0.0014-2.7 ng/mL E2 (MMV-Luc), 0.03-28.8 ng/mL testosterone (TARM-Luc), 0.5-314.5 ng/mL progesterone (TM-Luc) and 4.5-181.2 ng/mL hydrocortisol (TGRM-Luc). Antagonist tests were carried out by incubating the test compounds with the relevant positive control. The hormone spiking concentrations used in the experiment were as follows: 0.5 ng/mL E2 (MMV-Luc), 2.9 ng/mL testosterone (TARM-Luc), 5 ng/mL progesterone (TM-Luc) and 100 ng/mL hydrocortisol (TGRM-Luc).

For RGAs, dose-response curves were fitted with SlideWrite Plus V6 software using the sigmoidal dose-response curve equation, Y=Bottom + (Top-Bottom)/(1+10(Log EC50-X)), here X is the logarithm of concentration, Y the response, and Bottom and Top are fixed to 0% and 100% respectively of the maximum response of the standard used in each test. Fold induction was measured by calculating the ratio of a response when compared with the negative control

(n-fold). The concentration that produced a 50% increase in maximal response (EC_{50}) was used as a measurement of the assays sensitivity. All values shown are expressed as mean ± standard deviation (SD).

Extraction recoveries were calculated based on standard curve equations, where the X value was calculated for a given Y value, response achieved from the assay. Calculated in this way concentration was divided by the value of spiking concentration before extraction and multiplied by 100 to achieve a % extraction recovery.