Supporting Information

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SI Materials and Methods

Synthetic Chemistry Experimental Methods. ***Chromatographic purification.*** Chromatographic purification was performed using prepacked silica gel cartridges. The Flashmaster (Argonaut Technologies) is an automated multiuser flash chromatography system that uses disposable, normal-phase, solid-phase extraction cartridges (2 g to 100 g). It provides quaternary on-line solvent mixing to enable gradient methods to be run. Samples are queued using the multifunctional open access software, which manages solvents, flow rates, gradient profile, and collection conditions. The system is equipped with a Knauer variable-wavelength UV detector and 2 Gilson FC204 fraction collectors, enabling automated peak cutting, collection, and tracking.

NMR. ^{1}H NMR spectra were recorded in either $CDCl_3$ or DMSO-d_6 on either a Bruker DPX 400 or Bruker Avance DRX or Varian Unity 400 spectrometer, all working at 400 MHz. The internal standard used was either tetramethylsilane or the residual protonated solvent at 7.25 ppm for $CDCl_3$ or 2.50 ppm for DMSO-d_6.

Mass-directed autopreparative HPLC. Mass-directed autopreparative HPLC was performed with Agilent 1100 Series LC/MSD hardware, using electrospray positive mode and running ChemStation 32 purification software. Column: Zorbax Eclipse XDB-C18 prep HT (dimensions, 212 × 100 mm; 5-μm packing), 20 mL/min solvent speed. Aqueous solvent was water plus 0.1% TFA. Organic solvent was MeCN plus 0.1% TFA. Specific gradient used: 1 min 70% water (0.1% TFA)/30% MeCN (0.1% TFA), increasing over 9 min to 5% water (0.1% TFA)/95% MeCN (0.1% TFA) to elute compounds.

Liquid chromatography/mass spectrometry system. The liquid chromatography/mass spectrometry (LC/MS) system used was as follows: column, 3.3 cm × 4.6 mm internal diameter, 3 μm ABZ+PLUS from Supelco; flow rate, 3 mL/min; injection volume, 5 μL; room temperature; UV detection range, 215–330 nm; solvent A, 0.1% formic acid plus 10 mM ammonium acetate; and solvent B, 95% acetonitrile + 0.05% formic acid. Gradients are shown in Table S1.

Differential scanning calorimetry. The differential scanning calorimetry (DSC) thermogram of crystalline 1-{[3-(4-{[(2*R*)-4-[5-fluoro-2-(methyloxy)phenyl]-2-hydroxy-4-methyl-2-(trifluoromethyl)pentyl]amino}-6-methyl-1*H*-indazol-1-yl)phenyl]carbonyl}-D-prolinamide was obtained using a TA Q1000 calorimeter, serial no. 1000–0126. The sample was weighed into an aluminium pan and a pan lid placed on top and lightly crimped without sealing the pan. The experiment was conducted using a heating rate of 10 °C min^{-1}.

Synthetic schemes. The *meta*-amide derivatives **6** and **10–12** were readily prepared from the racemic epoxide **16** (1) (Scheme S1). Thus, ytterbium triflate–catalyzed opening of this epoxide with the amino indazole benzyl ester **17,** followed by removal of the benzyl protection by hydrogenolysis, afforded the key intermediate racemic *meta*-carboxylic acid **9**. Amide bond formation via *O*-(7-azabenzotriazol-1-yl)-*N,N,N*≪,*N*≪-tetramethyluronium hexafluorophosphate (HATU)-mediated coupling of **9** with the requisite amine, followed by separation of isomers by chiral HPLC, afforded the required *meta*-amides **10** and **11**. GR agonist activity has been shown to reside predominantly in isomers having the *R* configuration at the hydroxyl-bearing stereogenic center, and asymmetric syntheses have subsequently been developed (1). The (3S)-2-pyrrolidinone amide **12** was tested as the *2*R*/*S* mixture. The truncated analogues **13–15** were prepared in a similar manner, starting from the simple achiral epoxide **18**.

Phenylmethyl 3-(4-amino-6-methyl-1H-indazol-1-yl)benzoate. 6-Methyl-1*H*-indazol-4-amine hydrochloride [may be prepared using the methodology described by Davies (2); 0.5 g, 2.7 mmol], phenylmethyl 3-iodobenzoate (0.9 g, 2.6 mmol), copper (I) iodide (14 mg, 0.07 mmol), potassium carbonate (1.2 g, 8.68 mmol), and *trans*-*N,N*≪-dimethyl-1,2-cyclohexanediamine (20 mg, 0.14 mmol) were heated together in *N,N*-dimethylformamide (DMF) (5 mL) at reflux overnight. The mixture was poured into water (15 mL), and ethyl acetate was added to dissolve the resulting oil. The suspension was then filtered through Celite. The organic phase was separated, combined with a second ethyl acetate extract, washed successively with water and brine, and then dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (75 g) eluting with 1–5% gradient of ethyl acetate in dichloromethane (DCM) to give the *title compound* as a light-brown oil (0.3 g).

^{1}H-NMR: ($CDCl_3$, 400 MHz) δ 8.46 (t, 1 H), 8.10 (s, 1 H), 8.06 (m, 1 H), 7.96 (m, 1 H), 7.61 (t, 1 H), 7.49 (m, 2 H), 7.42 (m, 2 H), 7.38 (m, 1 H), 6.96 (s, 1 H), 6.31 (s, 1 H), 5.44 (s, 2 H), 4.15 (m, 2 H), 2.42 (s, 3 H).

Phenylmethyl 3-(4-{[4-[5-fluoro-2-(methyloxy)phenyl]-2-hydroxy-4-methyl-2-(trifluoromethyl)pentyl]amino}-6-methyl-1H-indazol-1-yl)benzoate. A solution of racemic 2-{2-[5-fluoro-2-(methyloxy)phenyl]-2-methylpropyl}-2-(trifluoromethyl)oxirane (**16**) (which may be prepared according to patent WO 04/063163; 350 mg, 1.2 mmol), in acetonitrile (2 mL) was added to a mixture of phenylmethyl 3-(4-amino-6-methyl-1*H*-indazol-1-yl)benzoate (357 mg, 1.0 mmol) and ytterbium (III) triflate (124 mg, 0.2 mmol). The mixture was stirred and heated to 85 °C under nitrogen in a greenhouse apparatus for 18 h, when the temperature was raised to 100 °C and the mixture heated under vigorous reflux for approximately a further 21 h. The mixture was cooled to room temperature and partitioned between DCM (50 mL) and aqueous sodium bicarbonate (50 mL). The aqueous layer was extracted again with DCM (50 mL), and the combined organic extracts were dried over anhydrous sodium sulfate and evaporated. The residue was purified by silica gel chromatography using a Flashmaster apparatus (50-g cartridge) eluting with a cyclohexane to 1:1 cyclohexane/ethyl acetate gradient over 40 min to give the *title compound* as a white solid (424 mg).

^{1}H-NMR: ($CDCl_3$, 400 MHz) δ 8.40 (t, 1 H), 8.04–8.07 (m, 1 H), 7.97 (s, 1 H), 7.91 (ddd, 1 H), 7.60 (t, 1 H), 7.46–7.49 (m, 2 H), 7.35–7.43 (m, 4 H), 7.17 (dd, 1 H), 6.91–6.99 (m, 2 H), 6.85 (dd, 1 H), 5.70 (broad s, 1 H), 5.42 (s, 2 H), 3.87 (s, 3 H), 3.35 (d, 1 H), 3.12 (d, 1 H), 2.88 (d, 1 H), 2.38 (s, 3 H), 2.28 (d,1 H), 1.46 (s, 3 H), 1.43 (s, 3 H).

***3-(4-{[4-[5-Fluoro-2-(methyloxy)phenyl]-2-hydroxy-4-methyl-2-(trifluoromethyl)pentyl]amino}-6-methyl-1H-indazol-1-yl)benzoic acid* (9)**. Phenylmethyl 3-(4-{[4-[5-fluoro-2-(methyloxy)phenyl]-2-hydroxy-4-methyl-2-(trifluoromethyl)pentyl]amino}-6-methyl-1*H*-indazol-1-yl)benzoate (2.33 g, 3.59 mmol) was suspended in ethanol (75 mL) and hydrogenated with vigorous stirring at 5 atmospheres at room temperature in the presence of 10% palladium on carbon (700 mg) for 16 h. The mixture was filtered through Celite and the filtrate evaporated to provide the *title compound* as a pale-yellow foam (1.85 g).

LC/MS: t_{RET} = 4.06 min; MH^+ = 560.

N-[(1R)-2-amino-1-methyl-2-oxoethyl]-3-(4-{[4-[5-fluoro-2-(methyloxy)-phenyl]-2-hydroxy-4-methyl-2-(trifluoromethyl)pentyl]amino}-6-methyl-1H-indazol-1-yl)benzamide. *N,N*-Diisopropylethylamine (DIPEA; 0.312 mL, 1.79 mmol) was added to a solution of 3-[4-

({4-[5-fluoro-2-(methyloxy)phenyl]-2-hydroxy-2,4-dimethylpentyl}amino)-6-methyl-1*H*-indazol-1-yl]benzoic acid (200 mg, 0.357 mmol) and HATU (135.7 mg, 0.357 mmol) in DMF (2.5 mL) and the solution stirred at room temperature for 5 min. Alaninamide hydrochloride (111.2 mg, 0.893 mmol) was added and the mixture stirred at room temperature overnight and then partitioned between 2 M HCl (50 mL) and ethyl acetate (50 mL). The organic layer was separated, washed with aqueous sodium bicarbonate (50 mL), dried over anhydrous sodium sulfate, and evaporated to give crude product, which was purified by mass-directed autopreparation (system B) to give the *title compound* (95.8 mg).

LC/MS: t_{RET} = 3.65 min; MH^+ = 630.

This mixture of diastereomers was resolved by chiral HPLC on a 2 in × 20 cm Chiralpak AD column eluted with heptane/ethanol 2:8 with a flow rate of 75 mL/min to provide diastereomer A (42.4 mg) and diastereomer B (45.2 mg).

Diastereomer A (compound **10**). Analytic chiral HPLC (25 × 0.46-cm Chiralpak AD column, heptane/ethanol 2:8 eluting at 1 mL/min): t_{RET} = 5.1 min.

LC/MS: t_{RET} = 3.66 min; MH^+ = 630.

Diastereomer B. Analytic chiral HPLC (25 × 0.46-cm Chiralpak AD column, heptane/ethanol 2:8 eluting at 1 mL/min): t_{RET} = 10.9 min.

LC/MS: t_{RET} = 3.66 min; MH^+ = 630.

1-{[3-(4-{[4-[5-Fluoro-2-(methyloxy)phenyl]-2-hydroxy-4-methyl-2-(trifluoromethyl)pentyl]amino}-6-methyl-1H-indazol-1-yl)phenyl]carbonyl}-D-prolinamide. Prepared similarly to D-alaninamide (**10**) from 3-[4-({4-[5-fluoro-2-(methyloxy)phenyl]-2-hydroxy-2,4-dimethylpentyl}amino)-6-methyl-1*H*-indazol-1-yl]benzoic acid and D-prolinamide.

LC/MS: t_{RET} = 3.63 min; MH^+ = 656.

Of this mixture of diastereomers, 118.8 mg was resolved by chiral HPLC on a 2 in × 20 cm Chiralpak AD column eluted with heptane/ethanol 1:9 with a flow rate of 75 mL/min to provide diastereomer A (59 mg) and diastereomer B (61 mg).

*Diastereomer A (2*R *isomer, compound* **11**). Analytic chiral HPLC (25 × 0.46-cm Chiralpak AD column, heptane/ethanol 1:9 eluting at 1 mL/min): t_{RET} = 8.4 min.

LC/MS: t_{RET} = 3.63 min; MH^+ = 656.

A sample of this diastereomer from a similar preparation was recrystallized from toluene to give very fine needles, melting point onset (DSC) 114 °C.

*Diastereomer B (2*S *isomer).* Analytic chiral HPLC (25 × 0.46-cm Chiralpak AD column, heptane/ethanol 1:9 eluting at 1 mL/min): t_{RET} = 22.4 min.

LC/MS: t_{RET} = 3.63 min; MH^+ = 656.

3-(4-{[4-[5-Fluoro-2-(methyloxy)phenyl]-2-hydroxy-4-methyl-2-(trifluoromethyl)pentyl]amino}-6-methyl-1H-indazol-1-yl)-N-[(3S)-2-oxo-3-pyrrolidinyl]benzamide (**12**). Prepared similarly to D-alaninamide (**10**) from 3-[4-({4-[5-fluoro-2-(methyloxy)phenyl]-2-hydroxy-2,4-dimethylpentyl}amino)-6-methyl-1*H*-indazol-1-yl]benzoic acid and (3*S*)-3-amino-2-pyrrolidinone.

LC/MS: t_{RET} = 3.53 min; MH^+ = 642.

1,1,1-Trifluoro-4-[5-fluoro-2-(methyloxy)phenyl]-4-methyl-2-{[(6-methyl-1-phenyl-1H-indazol-4-yl)amino]methyl}-2-pentanol. Prepared from racemic 2-{2-[5-fluoro-2-(methyloxy)phenyl]-2-methylpropyl}-2-(trifluoromethyl)oxirane and 6-methyl-1-phenyl-1*H*-indazol-4-amine, as described in patent WO 06/108699.

LC/MS: t_{RET} = 4.20 min; MH^+ = 516.

This racemic material (37 mg) was resolved by chiral HPLC on a 25 × 2-cm Chiralcel OD column eluted with heptane/ethanol 4:1 with a flow rate of 15 mL/min to provide enantiomer A (9.4 mg) and enantiomer B (9.8 mg).

Enantiomer A. Analytic chiral HPLC (25 × 0.46-cm Chiralcel OD-H column, heptane/ethanol 4:1 eluting at 1 mL/min): t_{RET} = 5.50 min.

LC/MS: t_{RET} = 4.13 min; MH^+ = 516

Enantiomer B (compound **6**). Analytic chiral HPLC (25 × 0.46-cm Chiralcel OD-H column, heptane/ethanol 4:1 eluting at 1 mL/min): t_{RET} = 6.64 min.

LC/MS: t_{RET} = 4.15 min; MH^+ = 516.

Phenylmethyl 3-(6-methyl-4-{[3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propyl]amino}-1H-indazol-1-yl)benzoate. A mixture of phenylmethyl 3-(4-amino-6-methyl-1*H*-indazol-1-yl)benzoate (166 mg, 0.46 mmol), 2,2-bis(trifluoromethyl)oxirane (100 mg, 0.55 mmol), and ytterbium(III) triflate (50 mg, 0.09 mmol) in acetonitrile (2.5 mL) was heated with stirring in a Reacti-Vial at 85 °C for 6 h. The solvent was evaporated, and the dark brown residue was dissolved in DCM and purified by silica gel chromatography on a Flashmaster apparatus (70-g cartridge) using a 0–50% ethyl acetate–cyclohexane gradient over 40 min. Evaporation of the product containing fractions afforded the *title compound* (145 mg).

LC/MS: t_{RET} = 4.08 min; MH^+ = 538.

3-(6-Methyl-4-{[3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propyl]amino}-1H-indazol-1-yl)benzoic acid. Phenylmethyl 3-(6-methyl-4-{[3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propyl]amino}-1*H*-indazol-1-yl)benzoate (144 mg, 0.27 mmol) was dissolved in methanol (20 mL) and hydrogenolyzed at room temperature and atmospheric pressure using an H-cube apparatus. Evaporation of the solvent left the *title compound* (120 mg).

LC/MS: t_{RET} = 3.58 min; MH^+ = 448.

N-methyl-3-(6-methyl-4-{[3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propyl]amino}-1H-indazol-1-yl)benzamide (**15**). A solution of HATU (38.1 mg, 0.1 mmol) in DMF (0.1 mL) was added to a solution of 3-(6-methyl-4-{[3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl-)propyl]amino}-1*H*-indazol-1-yl)benzoic acid (44.7 mg, 0.1 mmol) and DIPEA (30 μL, 0.3 mmol) in DMF (0.2 mL) and the mixture shaken for 5 min. The mixture was then added to a solution of methylamine (3.1 mg, 0.1 mmol) in DMF (0.2 mL), and after shaking for 5 min the mixture was left at room temperature overnight. The solvent was then removed on a vacuum centrifuge, and the residue was dissolved in 1:1 DMSO/methanol (0.5 mL) and purified by mass-directed autopreparation. Product-containing fractions were combined and evaporated to give the *title compound* (2.46 mg).

LC/MS: t_{RET} = 3.39 min; MH^+ = 461.

N-[(1*R*)-2-amino-1-methyl-2-oxoethyl]-3-(6-methyl-4-{[3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propyl]amino}-1H-indazol-1-yl)benzamide (**13**). To a solution of 3-(6-methyl-4-{[3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propyl]amino}-1H-indazol-1-yl)benzoic acid (1.05 g, 2.347 mmol) in DMF (22 mL) stirred under nitrogen at room temperature was added HATU (0.892 g, 2.347 mmol), and the reaction mixture was stirred at 22 °C for 20 min. Then DIPEA (1.435 mL, 8.22 mmol) and D-alaninamide hydrochloride (0.322 g, 2.58 mmol) in DMF (8 mL) were added, and the reaction mixture was stirred at 22 °C for a further 17 h when LC/MS showed the reaction to be complete. The solvent was evaporated, and the residue was partitioned between water and DCM. The organic phase was washed with water, dried by passage through a hydrophobic frit, and the solvent was evaporated to give a yellow oil. This crude product was purified first by silica gel chromatography eluting with a 0–25% methanol–DCM gradient to give impure material, which was then repurified by silica gel chromatography using a 0–15% methanol (+1% triethylamine)–DCM gradient to give the *title compound* as a yellow gum (1.05 g).

LC/MS: t_{RET} = 3.17 min; MH^+ = 518.

1-{[3-(6-Methyl-4-{[3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propyl]amino}-1H-indazol-1-yl)phenyl]carbonyl}-D-prolinamide (**14**). Prepared similarly to compound (**13**) from 3-(6-methyl-4-{[3,3,3-trifluoro-2-hydroxy-2-(trifluoromethyl)propyl]amino}-1*H*-indazol-1-yl)benzoic acid and D-prolinamide.

LC/MS: t_{RET} = 3.09 min; MH^+ = 544.

Pharmacokinetic Studies. Rat pharmacokinetic studies were performed at GlaxoSmithKline Medicines Research Center (Stevenage, Herts, United Kingdom) under the licence restrictions imposed by the United Kingdom Home Office. The pharmacokinetics of compounds **11** and **14** were studied using adult male Sprague-Dawley rats (Charles River Laboratories). The dose formulation for i.v. administration of compound **11** and for i.v. and oral administration of compound **14** was 10% DMSO/50% PEG 200/40% water (vol/vol) administered at 1 mg/kg. For oral administration of compound **11**, the compound was formulated as a suspension in 1% DMSO/99% 0.5% (wt/vol) methyl cellulose (aq) administered at 1 mg/kg. Test articles were administered discretely to ≥2 rats and serial blood samples collected into heparin-coated tubes at time points up to 12 h after administration. Plasma samples were harvested and stored at −20 °C before analysis.

Dog pharmacokinetic studies were performed at GlaxoSmithKline, The Frythe (Welwyn, Herts, United Kingdom) under the licence restrictions imposed by the United Kingdom Home Office. Compound **11** was studied using adult male beagles sourced from within GlaxoSmithKline. Three male dogs received a 30-min i.v. infusion of compound **11** at a nominal dose level of 0.5 mg/kg, followed 1 week later by an oral dose at a nominal dose level of 0.5 mg/kg administered as a crossover design. The i.v. dose was formulated as a solution in 10% DMSO/50% PEG 200/40% water at a nominal dose concentration of 0.2 mg/mL. The oral dose was formulated as a suspension in 0.5% (wt/vol) methyl cellulose in water at a nominal concentration of 1 mg/mL. Serial blood samples were collected from the cephalic vein in to heparinized tubes up to 12 h after dose. Plasma samples were harvested and stored at −20 °C before analysis.

Plasma samples were extracted using protein precipitation with acetonitrile using a 96-well filter plate (Whatman). The samples were drawn through the 96-well filter plate using a vacuum and the filtered extracts collected and diluted. The sample extracts were analyzed by LC-MS/MS analysis. HPLC separation was performed on a HP1100 binary pump and vacuum degasser (Agilent Technologies), HTS PAL autosampler (LEAP Technologies), and column-switching valve (Valco Instruments) using a Luna C18 5 cm × 2.1 mm column, 5-μm particle size (Phenomenex) operating at 40 °C. Samples were eluted at 800 μL/min using a gradient mobile phase consisting of 0.1% formic acid (vol/vol) in water and 0.1% formic acid (vol/vol) in acetonitrile. Test article detection was performed on a Sciex API4000 mass spectrometer (Applied BioSystems) using TurboIonSpray. Plasma standard curves were generated by plotting peak area ratio of analyte to internal standard against nominal concentrations. The acceptance criterion for inclusion of a calibration standard was ±20% of the nominal value. Pharmacokinetic parameters were determined using an in-house-developed macro operating within Microsoft Excel. Calculations were performed using noncompartmental analysis and using standard formula.

Biological Assays. ***GR binding assay.*** The ability of compounds to bind to the GR was determined by assessing their ability to compete with either an Alexa 555 fluorescently labeled (assay format A) or a Cy3b fluorescently labeled (assay format B) dexamethasone. Compounds were solvated and diluted in DMSO and transferred directly into assay plates. Fluorescent dexamethasone and a partially purified full-length GR were added to the plates, together with buffer components to stabilize the GR protein (Panvera peptide [assay format A] or in-house stabilization peptide [assay format B]) and incubated at room temperature for 2 h in the dark. Binding of each compound was assessed by analyzing the displacement of fluorescent ligand by measuring the decrease in fluorescence polarization signal from the mixture. An appropriate reader was used to assess the displacement, with filters set for 535 nM excitation and 590 nM emission. Dose–response curves were constructed, from which pIC_{50} values were estimated.

GR-mediated TR of NF-κB activity. Human A549 lung epithelial cells were engineered to contain a secreted placental alkaline phosphatase gene under the control of the distal region of the NF-κB–dependent ELAM promoter, as previously described by Ray et al. (3). Compounds were solvated and diluted in DMSO and transferred directly into assay plates, such that the final concentration of DMSO was 0.7%. After the addition of cells (10×10^3 per well), 384-well plates were incubated for 1 h before the addition of 3.2 ng/mL human recombinant TNFα. After continued incubation for 16 h, alkaline phosphatase activity was determined by measuring the change in optical density at 405 nM with time after the addition of 0.35 volumes of assay buffer (2 mg/mL *p*-nitrophenylphosphate dissolved in 1 M diethanolamine, 0.28 M NaCl, and 0.5 mM $MgCl_2$). Dose–response curves were constructed, from which pIC_{50} values were estimated and from which maximal responses are calculated relative to dexamethasone (100%).

Glucocorticoid-mediated gene TA: MMTV assay. Human A549 lung epithelial cells were engineered to contain a Renilla luciferase reporter gene construct under the control of MMTV. MMTV sequences include glucocorticoid responsive elements, which confer GR dependence on luciferase gene expression as a surrogate of GR-dependent TA. Compounds were solvated and diluted in DMSO and transferred directly into assay plates, such that the final concentration of DMSO was 0.7%. A 70% confluent T225 flask of A549 MMTV cells was harvested and diluted to 0.16×10^6/mL. Cell solution (70 μL) was dispensed to each well of white tissue culture–treated Nunc 384-well plates, containing compound at the required concentration. Plates were incubated for 6 h at 37 °C, 95% humidity, and 5% CO_2. After incubation, luciferase activity was quantified by measurement on the ViewLux imager, after the addition of 10 μL of Renilla luciferase substrate. Renilla luciferase was prepared according to the manufacturer's guidelines. Dose–response curves were constructed, from which pEC_{50} values were estimated and from which maximal responses were calculated relative to dexamethasone (100%).

Assay for GR MMTV antagonist activity. Human A549 lung epithelial cells were engineered to contain a Renilla luciferase reporter gene construct under the control of MMTV. MMTV sequences include glucocorticoid responsive elements, which confer GR dependence on luciferase gene expression as a surrogate of GR-dependent TA. Compounds were solvated and diluted in DMSO and transferred directly into assay plates, such that the final concentration of DMSO was 0.7%. A 70% confluent T225 flask of A549 MMTV cells was harvested and diluted to 0.16×10^6/mL. An appropriate EC_{80} of cortisol was added to the cell solution. Cell solution (70 μL) was dispensed to each well of white tissue culture–treated Nunc 384-well plates, containing compound at the required concentration. Plates were incubated for 24 h at 37 °C, 95% humidity, and 5% CO_2. After incubation, luciferase activity was quantified by measurement on the ViewLux imager, after the addition of 10 μL of Renilla luciferase substrate. Renilla luciferase was prepared according to the manufacturer's guidelines. Dose–response curves were constructed, from which pIC_{50} values were estimated and from which maximal responses were calculated relative to mifepristone (RU-486) (100%).

Assay for AR agonist activity. CV-1 cells were transiently transfected with Fugene-6 reagent according to the manufacturer's protocol. Briefly, a T175 flask of CV-1 cells at a density of 80% confluency was transfected with 25 μg of mix DNA and 75 μL of Fugene-6. The DNA mix (1.4 μg pAR, 2.5 μg pMMTV luciferase, and 18.75 μg pBluescript) was incubated with Fugene-6 in 5 mL Opti-

MEM-1 for 30 min and then diluted up to 20 mL in transfection media (DMEM containing 1% HyClone, 2 mM L-glutamine, and 25 mM Hepes) before addition to the cells. After 24 h, cells were washed with PBS, detached from the flask using 0.25% trypsin, and counted using a Sysmex KX-21N. Cells were either frozen or processed immediately. In either case, transfected cells were diluted in assay media (DMEM containing 5% HyClone and 2 mM L-glutamine), and 5×10^3 cells were dispensed to each well of white Nunc 384-well plates, containing compounds at the required concentration. After 24 h, 10 μL of Steady-Glo was added to each well of the plates. Plates were incubated in the dark for 10 min before being read on a Viewlux reader. Dose–response curves were constructed, from which pEC_{50} values were estimated.

Assay for AR binding activity. Briefly, 175 nM MBP-hAR-LBD (in-house protein), 1 nM AR red Fluoroprobe (Invitrogen), and 2 mM DTT were dissolved and mixed in the AR binding buffer [50 mM Tris (pH 7.5), 100 mM ammonium sulfate, 20% glycerol, 3% xylitol, and 5 mM CHAPS]. Ten microliters of the mix was dispensed to each well of Greiner low-volume 384-well plates, containing compounds at the required concentration. The plates were spun for 1 min at 200 $\times$ *g*, covered to protect the reagents from light, and then incubated at room temperature for $\approx$2 h. Plates were read on an Acquest using a 485-nm excitation filter, a 530-nm emission interference filter, and a 505-nm Dichroic mirror. Dose–response curves were constructed, from which pIC_{50} values were estimated.

Assay for ERα binding activity. Briefly, 4 nM ERα (in-house protein) and 1.5 nM Fluormone EL-Red (Invitrogen) were dissolved and mixed in ER binding buffer [50 mM Mops (pH 7.5), 50 mM NaF, 2.5 mM CHAPS, and 5 mM DTT]. Ten microliters of the mix was dispensed to each well of Greiner low-volume 384-well plates, containing compounds at the required concentration. The plates were spun for 1 min at 200 $\times$ *g*, covered to protect the reagents from light, and then incubated at room temperature for 2 h. Plates were read on an Acquest using a 530–25-nm excitation and 580–10-nm emission interference filter and a 561-nm dichroic mirror. Dose–response curves were constructed, from which pIC_{50} values were estimated.

Assay for ERβ binding activity. Briefly, 6 nM ERβ (in-house protein) and 1.5 nM Fluormone EL-Red were dissolved and mixed in ER binding buffer [50 mM Mops (pH 7.5), 50 mM NaF, 2.5 mM CHAPS, and 5 mM DTT]. Ten microliters of the mix was dispensed to each well of Greiner low-volume 384-well plates, containing compounds at the required concentration. The plates were spun for 1 min at 200 $\times$ *g*, covered to protect the reagents from light, and then incubated at room temperature for 2 h. Plates were read on an Acquest using a 530–25-nm excitation and 580–10-nm emission interference filter and a 561-nm dichroic mirror. Dose–response curves were constructed, from which pIC_{50} values were estimated.

Assay for MR agonist activity. Monkey kidney CV-1 cells were transiently transfected with Fugene-6 reagent according to the manufacturer's protocol. Briefly, a T225 flask of CV-1 cells at a density of 80% confluency was transfected with 40 μg of mix DNA and 120 μL of Fugene-6. The DNA mix (4.8 μg pMR, 9.6 μg pMMTV luciferase, 9.6 μg pPGC1, and 16 μg pBluescript) was incubated with Fugene-6 in 5 mL Optimem for 30 min and then diluted up to 30 mL in transfection media (DMEM containing 1% HyClone and 2 mM L-glutamine) before addition to the cells. After 24 h, cells were washed with PBS, detached from the flask using 0.25% of trypsin, and counted using a Sysmex KX-21N. Cells were either frozen or processed immediately. In either case, transfected cells were diluted in assay media (DMEM containing 1% HyClone and 2 mM L-glutamine) at 100 cells/μL. Fifty microliters of suspension cells were dispensed to each well of white Nunc 384-well plates, containing compound at the required concentration. After 24 h, 10 μL of Steady-Glo was added to each well of the plates. Plates were incubated in the dark for 10 min before being read on a Viewlux reader. Dose–response curves were constructed, from which pEC_{50} values were estimated.

Assay for MR antagonist activity. Monkey kidney CV-1 cells were transiently transfected with Fugene-6 reagent according to the manufacturer's protocol. Briefly, a T225 flask of CV-1 cells at a density of 80% confluency was transfected with 40 μg of mix DNA and 120 μL of Fugene-6. The DNA mix (4.8 μg pMR, 9.6 μg pMMTV luciferase, 9.6 μg PGC1, and 16 μg pBluescript) was incubated with Fugene-6 in 5 mL Optimem for 30 min and then diluted up to 30 ml in transfection media (DMEM containing 1% HyClone and 2 mM L-glutamine) before addition to the cells. After 24 h, cells were washed with PBS, detached from the flask using 0.25% of trypsin, and counted using a Sysmex KX-21N. Cells were either frozen or processed immediately. In either case, transfected cells were diluted in assay media (DMEM containing 1% HyClone and 2 mM L-glutamine) at 100 cells/μL, and aldosterone was added to the cells at the final concentration of 0.2 nM. Fifty microliters of suspension cells were dispensed to each well of white Nunc 384-well plates, containing compounds at the required concentration. After 24 h, 10 μL of Steady-Glo was added to each well of the plates. Plates were incubated in the dark for 10 min before being read on a Viewlux reader. Dose–response curves were constructed, from which pIC_{50} values were estimated.

Peptide binding assay. N-terminally biotinylated NR Box peptides derived from PGC-1 (DGTPPPQEAEEPSLLKKLLLAPANT), RIP140 (LERNNIKQAANNSLLLHLLKSQTIP), CBP (ADPEKRKLIQQQLVLLLHAHKCQ), SRC-1 (LDASKKKESKDHQLLRYLLDKDEKD), p300 (GISPLKPGTVSQQALQNLLRTLRSP), TIF-2 (DGQSRLHDSKGQTKLLQLLTTKSDQ), and the related sequence from NCoR (GHSFADPASNLGLEDIIRKALMGSF) were individually coupled to the surface of avidin-coated fluorescently distinct microspheres. These were incubated with Alexa 532–labeled GR LBD (containing an F602S mutation to enhance stability) in the presence of 40 μM of each compound for 4 h and the binding of the protein to each peptide assessed using flow cytometry.

Computational methods. All molecular modeling images were prepared using SYBYL, version 7.3. Two computational methods, AlleGrow and FLO+ molecular modeling systems, were used to assist the design process. The GR ligand-binding site protein model, previously described (4), was used as a starting point, with compound **6** placed into the site using the FLO+ "mcdock" procedure (500 iterations), allowing flexibility for all residues lining the binding pocket and creating zero bonds between the central hydroxy group and the Asn-564 side chain carbonyl and also from the ligand pyrazole to Gln-570 side chain NH. The receptor-positioned compound **6** was modified to introduce a "meta" amide group with an AlleGrow growth point in the *trans* position filling 1 valency of the amide nitrogen. The amide group, with growth point, was rotated in the plane of the phenyl ring to provide both reasonable starting points. The "grow" facility of AlleGrow was used, with multiple runs directing growth with targets of 4, 5, 6, 7, 8, 9, and 10 atoms. Output from these separate runs was combined to produce several thousand virtual compounds. These were scored within the protein site using the FLO+ scoring accessed through the AlleGrow utility "postgrow+." This uses the FLO+ "dockmin+" minimization procedure, with all virtual ligands finally binned according to predicted binding affinity. Those in low-affinity bins were discarded, whereas the remainder were evaluated visually within the site to make a selection of suitable compounds for synthesis. Thirty compounds, for which the required amines were readily available, were identified for synthesis.

Crystallographic methods. Crystallographic parameters are shown in Table S2. The GR LBD protein was the same F602Y and

C638G construct used for fluticasone furoate and was purified and complexed with a 12-residue TIF2 coactivator peptide using the published protocol (5). Compound **11** was crystallized in 28% PEG 5,000 monomethyl ether and 0.1 M Mes 6.5, and the crystals formed over 1 month. The well condition was the cryoprotectant solution for freezing. These crystals were hexagonal and small, with the longest edge being 20 μm. The initial crystals of GR LBD complexed with compound **13** were obtained from the following crystallization conditions: 0.1 M Mes 6.5 and 1.6 M magnesium sulfate. The crystals were optimized after a second preparation of protein to 2.0 M magnesium sulfate with 0.1 M Mes 6.5 and the additive β-hexylglucoside, and the crystals formed overnight. These crystals were quite large and reproducible, with the longest edge being 400 μm. Both data sets were reduced and scaled using HKL2000. The structure for compound **11** was solved by molecular replacement using *Amore* and refined using the program *CNX* and *Quanta* as the building program. The crystal structure for compound **13** was solved by molecular replacement using *Phaser* and refined with *Refmac*, and *Coot* was used as the building program. The crystal structures have been deposited in the RCSB as 3K23 for the D-prolinamide **11** and 3K22 for the D-alaninamide **13**.

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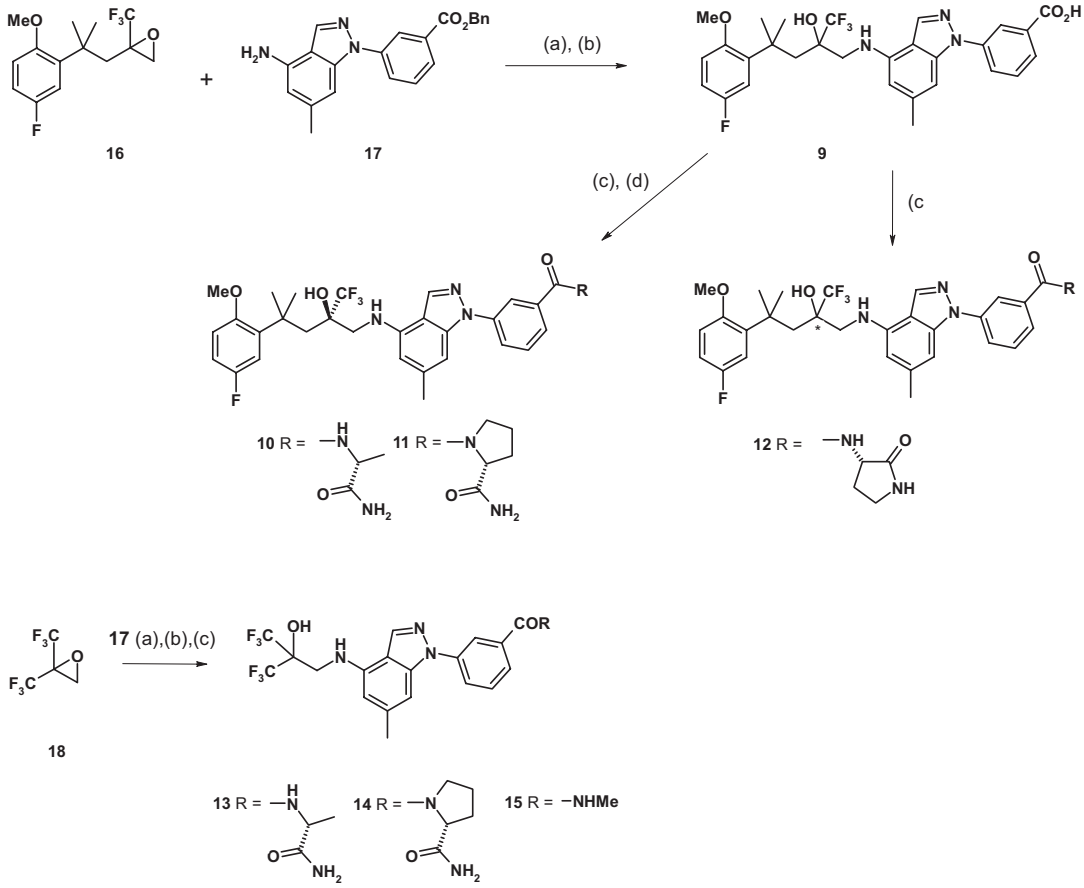

Scheme S1. (*a*) Yb(OTf)$_3$, CH_3CN, 85-100° C. (*b*) H_2, Pd/C, EtOH or MeOH. (*c*) Amine, HATU, DIPEA, DMF, room temperature. (*d*) Isomer separation by chiral HPLC.

Table S1. Gradients

Time	A%	B%
0.00	100	0
0.70	100	0
4.20	0	100
5.30	0	100
5.50	100	0

Table S2. Crystallographic parameters

Parameter	GR LBD/compound **13**	GR LBD/compound **11**
Crystallographic data		
Space group	$P6_1$	C2
a, b, c (Å)	127.83, 127.83, 76.54	184.9, 65.9, 71.5
a, b, g (°)	90, 90, 120	90, 103.6, 90
Molecules in ASU	2	3
Resolution range (Å)	50–2.50	20–3.0
Completeness (final shell)	88.8 (57.5)	99.1 (93.2)
I/σ (final shell)	37.5 (4.5)	15.8 (2.3)
R_{merge} % (final shell)	6.1 (38.2)	7.9 (38.1)
Refinement statistics		
Resolution range (Å)	20–2.50	20–3.0
% R_{free}	7	7
R_{cryst} (R_{free}) %	22.3 (27.3)	22.9 (28.9)
rmsd bonds (Å)	0.007	0.008
rmsd angles (°)	1.006	1.243