Title
A Novel Radioligand Reveals Tissue Specific Pharmacological Modulation of Glucocorticoid Receptor Expression with Positron Emission Tomography.

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ABSTRACT: The complexity of glucocorticoid receptor (GR) signaling cannot be measured with direct tissue analysis in living subjects, which has stilled our understanding of GR’s role in human physiology or disease and impeded the development of selective GR modulators. Herein, we report \( ^{18}\text{F}-5-(4\text{-fluorobenzyl})-10\text{-methoxy}-2,2,4\text{-trimethyl}-2,5\text{-dihydro-1H-chromeno}[3,4-f]\text{-quinoline (}\text{^{18}\text{F}}\text{-YJH08), a radioligand that enables noninvasive measurements of tissue autonomous GR expression levels in vivo with positron emission tomography (PET)}\). YJH08 potently binds GR (\( K_{i} \approx 0.4 \text{nM} \)) with \( \sim 100\text{-fold} \) selectivity compared to nuclear hormone receptors in the same subfamily. \( ^{18}\text{F}-\text{YJH08} \) was prepared via Cu(OTf)\(_2\)(py)\(_4\)-mediated radiofluorination of an arylboronic acid pinacol ester with \( \sim 12\% \) decay corrected radiochemical yield from the starting \( ^{18}\text{F}-\text{fluoride ion} \). We applied treatment with the tissue-wide GR agonist dexamethasone and adrenalectomy and generated an adipocyte specific GR knockout mouse to show that \( ^{18}\text{F}-\text{YJH08} \) specifically binds GR in normal mouse tissues, including those for which aberrant GR expression is thought to drive severe diseases (e.g., brain, adipose tissue, kidneys). Remarkably, \( ^{18}\text{F}-\text{YJH08} \) PET also revealed that JG231, a potent and bioavailable HSP70 inhibitor, selectively degrades GR only in the adipose tissue of mice, a finding that foreshadows how GR targeted PET might be integrated into drug discovery to screen for selective GR modulation at the tissue level, beyond the historical screening that was performed at the transcriptional level. In summary, \( ^{18}\text{F}-\text{YJH08} \) enables a quantitative assessment of GR expression levels in real time among multiple tissues simultaneously, and this technology is a first step toward unraveling the daunting complexity of GR signaling and rationally engineering tissue specific therapeutic modulators in vivo.

The glucocorticoid receptor (GR) regulates an exceptionally diverse spectrum of physiological processes and is expressed in nearly every normal cell type.\(^{1,2}\) As a nuclear hormone receptor (NHR), GR regulates biology primarily through an inducible transcriptional mechanism, in which cytosolic corticosteroid agonists bind the isoform GR\( \alpha \) to trigger a conformational change that encourages nuclear translocation, homodimerization, and DNA binding to regulate transcription. Agonist bound GR\( \alpha \) can also regulate transcription through alternate mechanisms (e.g., tethering other transcription factors to DNA) or through nongenomic signaling. Moreover, other less abundant isoforms of GR, most notably the splice variant GR\( \beta \), may regulate additional dimensions of biology in a corticosteroid independent fashion. Further adding to the complexity, GR engages in both cell intrinsic and multiorgan endocrine signaling through secreted hormones (e.g., the hypothalamus–pituitary–adrenal axis).

Because of its seminal importance to the homeostasis of diverse cell types, dysregulation of GR signaling is accompanied by significant, and often highly debilitating, phenotypic changes. For instance, excess or deficient corticosteroid production and the concomitant dysregulation of cellular GR\( \alpha \) activity lead to Cushing syndrome or Addison’s disease, respectively. Moreover, tissue changes in GR expression—either losses or gains—have been implicated in the pathobiology of a wide spectrum of maladies, including mood disorders, glomerular diseases, and more recently, cancers.\(^{3}\)
Cell and genetically engineered animal models have been the mainstays for carefully articulating the above-mentioned features of GR (patho)biology. Fully understanding GR's role in human physiology and disease, as well as developing next generation therapeutics to more selectively modulate GR, requires new technologies that can safely probe GR signaling in living subjects. The lack of noninvasive biomarkers to interrogate GR signaling in humans has left the field to perform very suboptimal assays, for instance, analysis of GR and target gene expression levels from tissues collected at autopsy.1,2

With these considerations in mind, we hypothesized that direct measurement of GR expression levels in tissues with nuclear imaging could be an impactful starting point toward developing a complete repertoire of noninvasive tools to study the GR signaling axis. Radiolabeled steroid agonists targeting other NHRs in the same family have been implemented clinically with great success,6,7 which motivated us to design a radiolabeled small molecule probe targeting the ligand binding domain (LBD) of GR.

Herein, we describe the design, synthesis, and pharmacological evaluation of 18F-5-(4-fluorobenzyl)-10-methoxy-2,2,4-trimethyl-2,5-dihydro-1H-chromeno[3,4-f]quinoline (18F-YJH08), a small molecule radiotracer that measures GR expression levels in vivo with PET. YJH08 is a potent ligand for GR with weak agonist activity. A high yielding radiosynthesis of 18F-YJH08 was achieved using Cu(II) mediated fluorination of an arylboronic acid pinacol ester precursor. 18F-YJH08 specifically bound to GR in many normal mouse tissues in vivo, including several of great interest to the GR signaling community, including the brain, adipose tissue, and adrenals. Lastly, 18F-YJH08 PET was applied to study the anti-GR effects of the bioavailable HSP70 inhibitor JG231. Remarkably, GR degradation only occurred in adipose tissue. This unexpected finding underscores how 18F-YJH08 might be applied to identify tissue specific GR modulators early in the drug discovery process, an important unmet clinical need owing to the intense side effects associated with broad spectrum GR modulators (e.g., the semisynthetic corticosteroid dexamethasone).

RESULTS AND DISCUSSION

Design, Synthesis, and in Vitro Characterization of YJH08. The abundance of literature reporting suboptimal imaging findings with radiolabeled corticosteroids suggested to us that we ought to consider nonsteroidal structures with superior pharmacokinetics.8,9 Agonists generally bind to NHRs with higher affinity compared to antagonists, and highly potent and specific synthetic agonists built on benzopyrano[3,4-f]quinolines (Figure 1) were previously shown to be pharmacologically active and well tolerated in animals.10,11 Therefore, we chose to design a radioligand for GR based on this structural motif.

Prior medicinal chemistry has shown that the C5 position on the benzopyrano[3,4-f]quinoline scaffold can tolerate modification without diminishing potency and selectivity for GR, whereas the C10 position is less accepting of manipulation.12,13 On this basis, we envisioned installing a fluorine atom on the C5 position of the core scaffold. Since 18F-alkyl fluorides can rapidly radio-defluorinate in vivo, which results in high bone uptake of the fluoride anion, we chose to couple fluorine to an aromatic ring, a more metabolically stable approach. Retrosynthetically, we anticipated that fluorine-18 could be introduced on the aryl ring through one of several reported copper mediated radiofluorination chemistries.14,15

The synthesis of YJH08 began by preparing the methyl acetal 1 in six steps following literature precedent (Scheme 1, see also Supporting Information Methods).16 To avoid undesired oxidative side products during fluorination chemistry, the secondary amine on the benzopyrano[3,4-f]quinoline scaffold was protected with Boc2O to confer 2. A Grignard reaction with 4-bromobenzylmagnesium bromide was applied to install an aromatic moiety on the C5 position (3). A Stille-type coupling was then invoked to convert the aryl bromide to an arylboronic acid pinacol ester (4). This functional group was desired as it has been successfully used as a substrate for regioselective copper mediated radiofluorination reactions.17,18 Satisfyingly, we were able to convert this molecule to the aryl fluoride 5 at 68% yield within 2 h at 120 °C using Cu(OTf)2(Py)4, pyridine, KF, and K2CO3. The Boc group was then efficiently removed in 30 min with 50% TFA in DCM to confer 6. In summary, the overall yield of 4 was 25%, and the yield of 6 was 15%.

Next, the affinity of YJH08 for GR and the other type 1 subfamily 3 nuclear hormone receptors was evaluated using 3H-dexamethasone (Table S1 and Figure S1). We conducted cell-based studies to determine if YJH08 modulates GR activity. Real time PCR showed that YJH08 (10 μM, 6 h exposure) elevated the levels of two GR target genes (GILZ, FKBP5) in DU145, a GR positive human prostate cancer cell line (Figure 2B). The induction per gene was lower than what was observed for dexamethasone (1 μM, 6 h exposure), and one gene, Per1, was induced by dexamethasone but not YJH08. Overall, these data suggest that YJH08 may be a mild GR agonist.

Radioisynthesis of 18F-YJH08. A focused screen of reaction conditions revealed that 18F-Boc-YJH08 could be efficiently synthesized by hand in 20 min using K2CO3/KOTf as the base to mobilize 18F-fluoride anions from a QMA column (4.7 ± 0.8%, see Table S2, Figure S2, and Scheme 2). We also found that this reaction was amenable to automation using an ELIXYS FLEX/CHEM synthesis module, and HPLC analysis of the reaction progress showed that the reaction produces one major radioactive peak corresponding to 18F-Boc-YJH08 (Figure 3A). After purification, the Boc protecting group was removed with 50% TFA in DCM to confer 18F-YJH08 in 12.4 ± 3% decay corrected radiochemical yield (Figure 3B and Figure S3). The molar activity of 18F-YJH08 was calculated to be 0.042 ± 1 Ci/μmol over four independent radiosyntheses (Figure S4). The partition coefficient (log P) of 18F-YJH08 was assessed in instant thin layer chromatography and calculated to be 2.78.
Scheme 1. Synthesis of YJH08

Reagents and conditions: (a) n-BuLi, (Boc)₂O, THF, −45 °C, 51%; (b) 0.25 M 4-Bromobenzylmagnesium bromide in anhydrous Et₂O, BF₃·Et₂O, anhydrous DCM, −15 °C, 73%; (c) Pd(dba)₂Cl₂·DCM, KOAc, B₂Pin₂, anhydrous dioxane, 100 °C, 68%; (d) Cu(OTf)₂(Py)₄, Py, KF, K₂CO₃, DMF, 120 °C, 68%; (e) 50% TFA in CH₂Cl₂, 40 °C, 30 min, 95%.

Table 1. A Summary of the Binding Affinity of YJH08 Compared to Conventional Agonists for Subfamily 3 Nuclear Hormone Receptors

| Ligand | GR (M) | AR (M) | PR (M) | ER (M) | MR (M) |
|--------|--------|--------|--------|--------|--------|
| Reference | 2.03 × 10⁻⁹ | 4.96 × 10⁻¹⁰ | 6.27 × 10⁻¹⁰ | 6.94 × 10⁻⁹ | 1.41 × 10⁻⁸ |
| YJH08 | 4.01 × 10⁻¹⁰ | 2.20 × 10⁻⁷ | 5.55 × 10⁻⁷ | 1.90 × 10⁻⁵ | 2.90 × 10⁻⁷ |

The competitive binding data are presented in Figure 2A and Figure S2. The data were fit using a one site $K_i$ and nonlinear regression with Prism software. The data are representative of two independent experiments.

**Figure 2.** YJH08 is a high affinity ligand for GR with weak agonist properties. (A) A ligand displacement curve showing YJH08 and dexamethasone displace ³H-dexamethasone in DU145 cells. The data were fit using a one site nonlinear regression, and the coefficient of determination ($R^2$) values are highlighted in the inset box. Additional competitive binding curves are presented in the Supporting Information. (B) Real time PCR data showing the relative induction of three GR target genes in DU145 cells by dexamethasone (1 μM) or YJH08 (10 μM). Cells were harvested for mRNA analysis after a 6 h exposure to drug or vehicle. The data, normalized to the ΔCt levels in vehicle treated cells, support an assessment that YJH08 may be a weaker GR agonist than dexamethasone. Statistical significance was assessed using an unpaired, two-sided Student’s t test. **P < 0.01, *P < 0.05.
18F-YJH08 Specifically Binds GR in Vivo and Measures Receptor Saturation by a Synthetic GR Agonist. To understand the biodistribution of 18F-YJH08 in vivo, we first conducted a dynamic PET scan over 60 min in immunocompetent intact male C57Bl6/J mice. Region of interest analysis was applied to generate time activity curves, which revealed several enlightening trends (Figure 4A,B and Figure S5). First, the radiotracer rapidly cleared from serum, and fitting the data with a two phase exponential decay curve estimated the fast and slow half-lives to be ∼21 s and ∼760 s, respectively. Moreover, liver uptake was prominent at very early time points post injection (0–300 s), with continuous washout from ∼300 to 3600 s. These data suggest a hepatobiliary mechanism of clearance. Consistent with this model, significantly lower radiotracer accumulation was observed in the kidneys from 0 to 3600 s. Radiotracer uptake in the bone was low and approximately equivalent to muscle, which suggests that the compound is stable to radiodefluorination in vivo.

Notably, we also observed clear radiotracer uptake in tissues for which aberrant GR expression is thought to drive disease
pathobiology. For instance, we observed high radiotracer uptake in the brain (Figure 4C), a significant finding as the neuroimaging community has long sought to develop a noninvasive tool to measure changes in GR expression thought to be occurring in the brains of individuals suffering from mood disorders (e.g., anxiety, depression). Moreover, high tracer uptake occurred in supraspinal brown adipose tissue in which GR expression is expressed and hypothesized to regulate seminal processes like thermogenesis and beiging (Figure 4D). Last, we performed a broader survey of radiotracer biodistribution by analyzing tissues post mortem. In general, numerous GR rich tissues had high radiotracer uptake, including the adrenals, lungs, and heart (Table 2).

We next tested if $^{18}$F-YJH08 accumulation in tissues is due to specific GR binding. First, biodistribution studies were conducted to determine if dexamethasone treatment lowered $^{18}$F-YJH08 uptake in normal mouse tissues due to competitive GR binding. Previous studies, including our own experience with a radiofluorinated corticosteroid termed $^{18}$F-GR02, have shown that it can be challenging to engage GR with a...
Table 2. Biodistribution Data Collected 30, 60, 90 min after Injection of $^{18}$F-YJH08 in C57Bl6/J Mice

| Tissue             | 30 min  | 60 min  | 90 min  |
|--------------------|---------|---------|---------|
| blood              | 0.85 ± 0.1 | 0.57 ± 0.02 | 0.49 ± 0.06 |
| brain              | 1.68 ± 0.14 | 1.33 ± 0.06 | 0.97 ± 0.03 |
| heart              | 3.04 ± 0.75 | 1.33 ± 0.21 | 0.92 ± 0.14 |
| lung               | 2.65 ± 0.19 | 2.02 ± 0.29 | 1.39 ± 0.1 |
| liver              | 11.03 ± 0.89 | 7.7 ± 0.69 | 8.43 ± 1.38 |
| spleen             | 1.59 ± 0.13 | 0.83 ± 0.02 | 0.74 ± 0.15 |
| pancreas           | 4.5 ± 0.15 | 2.63 ± 0.05 | 1.9 ± 0.41 |
| kidney             | 4.5 ± 0.5 | 2.35 ± 0.34 | 1.86 ± 0.26 |
| stomach            | 1.88 ± 0.96 | 1.63 ± 0.92 | 2.61 ± 1.54 |
| small intestine    | 19.05 ± 3.27 | 8.61 ± 2.68 | 7.67 ± 2.34 |
| large intestine    | 1.33 ± 0.35 | 1.23 ± 0.43 | 6.67 ± 4.73 |
| supraspinal BAT    | 14.41 ± 3.42 | 16.4 ± 4.93 | 14.58 ± 1.91 |
| muscle             | 1.49 ± 0.2 | 1.15 ± 0.03 | 0.85 ± 0.18 |
| bone               | 1.11 ± 0.15 | 0.61 ± 0.15 | 0.58 ± 0.16 |
| adrenal gland      | 20.02 ± 2.21 | 9.43 ± 0.08 | 5.72 ± 2.32 |

The data are represented as mean ± standard deviation of the % ID/g.

cointravenous injection of excess cold ligand to an extent that measurably alters tracer biodistribution in tissues. We proposed that the solubility limits of hydrophobic steroids and steroid-like molecules in intravenous formulations account for this observation, as we also found that administering a competitive ligand for GR via gavage hours to days in advance of the imaging study very effectively suppressed radiotracer uptake. With this experience in mind, we administered dexamethasone via oral gavage in two ways. One treatment arm of C57Bl6/J mice received dexamethasone once (50 mg/kg) at 4 h prior to $^{18}$F-YJH08 injection, and another cohort received the drug once daily (100 mg/kg) via oral gavage for 3 days prior to the radiotracer injection. Biodistribution studies showed that a single dose of dexamethasone reduced radiotracer uptake in the small intestine, while a higher dose administered over several days suppressed radiotracer uptake in many tissues compared to mice receiving vehicle (Figure 5A and Figures S6, S7). To confirm that high dose dexamethasone did not unexpectedly reduce GR expression levels, we examined GR expression in select tissues in which a significant degree of radiotracer suppression was observed. We found that GR expression levels were equivalent in several normal tissues from treated and untreated mice, which provides confidence that the suppression of radiotracer uptake was due to competitive GR binding (Figure S8). We next evaluated radiotracer uptake in adrenalectomized (adx) mice, as disruption of the HPA axis is well understood to ablate circulating corticosteroids while triggering normal cells to autonomously upregulate GR. $^{18}$F-YJH08 uptake was higher in the tissues of adx mice compared to intact mice (Figure 5A and Figure S9). Collectively, these data show that tissue uptake of the radiotracer is a reflection of GR abundance and occupancy by endogenous ligand.

On PET, visually obvious decreases in radiotracer uptake due to dexamethasone treatment (100 mg/kg) were observed in several tissues (e.g., brain, supraspinal brown adipose tissue, and kidneys, see Figure S5). Moreover, digital autoradiography was applied to compare the intensity of radiotracer binding in the brain sections from adx mice to those from intact mice treated with dexamethasone (Figure S5C). Comparison of the relative intensity of the pseudocolor showed that radiotracer binding was uniformly higher in adx mice, as expected. Radiotracer binding was high in the isocortex, a relatively large anatomical area known to have high GR expression in the mouse brain based on in situ hybridization data (Figure S10). GR is highly expressed in smaller structures within the brain, for example the hippocampal formation, although this was challenging to detect with the limited resolution of autoradiography. Studies in nonhuman primates or humans will be required to interrogate the regions of specific GR binding within complex organs like the brain.

Finally, we conducted an exploratory study to determine if genetic knockout of GR reduced $^{18}$F-YJH08 uptake. Mice bearingloxP sites flanking exon 3 of the Nr3c1 gene were crossed with mice bearing Cre recombinase under the control of the adiponectin promoter to induce adipocyte specific GR knockout. PET/CT data clearly showed that $^{18}$F-YJH08 uptake was significantly reduced in several fat depots within the GRlox/lox, Adipoq-Cre knockout mice, most obviously the supraspinal and scapular brown fat depots (Figure 5D). Moreover, overall radiotracer binding in the abdomen was significantly reduced in the knockout mice compared to wild type litter mate control, which is likely attributable to a loss of GR in fat depots as well as the greater omentum. To our knowledge, this is the first reported evidence of specific radioligand binding to GR in the adipose tissue.

$^{18}$F-YJH08 Reveals Tissue Specific Modulation of GR Expression by JG231, a Potent HSP70 Inhibitor. The limitations of broad-spectrum GR modulators like corticosteroids are well-known, and the severe side effects associated with their long-term use (e.g., diabetes mellitus, osteoporosis) motivated drug development campaigns starting in the 1990s to identify safer GR modulators that selectively modulate GR activity. We hypothesized that $^{18}$F-YJH08 PET could complement these ongoing efforts by revealing tissue specific drug–GR interactions. To test this concept, we evaluated the biodistribution of $^{18}$F-YJH08 in mice after treatment with JG231, a potent HSP70 inhibitor that we have shown can induce GR degradation (Figure 6A and B). JG231 has a well-defined pharmacokinetic profile, and our prior experience with this molecule showed it to have antitumor activity in prostate cancer mouse models. Using the same treatment strategy, mice were subjected to 4 mg/kg/day for 3 days intraperitoneally and subsequently treated with $^{18}$F-YJH08. On PET, the only visually obvious alterations in radiotracer distribution were noted in the supraspinal brown fat depot and in a portion of the abdomen that we interpreted to be the greater omentum (Figure 6C). In these regions, it appeared that JG231 suppressed radiotracer binding, perhaps indicative of GR degradation. Post mortem imaging of the supraspinal brown fat depot more clearly showed that JG231 dramatically reduced radiotracer binding compared to the same tissue harvested from mice treated with vehicle (Figure 6C). Biodistribution studies also suggested that JG231 most significantly induced GR degradation in brown fat (Figure 6D). Immunoblot studies corroborated the biodistribution data, showing approximately ~50% reduction in GR expression levels due to JG231 treatment in brown fat, and no change in GR levels from other tissues, for example, kidneys, liver, and muscle (Figure 6E and Figure S11). To our knowledge, this is first example of tissue specific GR degradation. More generally, these early data suggest that $^{18}$F-YJH08 PET could be applied to identify therapies with unexpected tissue tropism or prospectively engineer desirable tissue specificity into experimental GR modulators.
CONCLUSIONS

Here, we report the design, synthesis, and characterization of 18F-YJH08, a first in class radioligand to measure GR expression noninvasively with PET. In vivo studies showed that 18F-YJH08 specifically binds to virtually all normal tissues in mice, including tissue compartments like the brain for which the imaging community has long desired biomarkers to study the GR signaling axis. Moreover, the level of GR specific binding in tissues observed with 18F-YJH08 stands out compared to previously reported biodistribution data for radiotracers targeting GR, including nonsteroidal agonists.24 We further show the radiotracer can be used to study the pharmacology of GR modulators, which led to the unexpected finding of tissue specific anti-GR activity for an inhibitor of HSP70, a protein expressed in virtually every tissue in the body. Overall, 18F-YJH08 provides an unprecedented multiorgan view of GR expression in real time, and its clinical translation will likely open...
new frontiers in our understanding of the (patho)biology of GR in humans.

**EXPERIMENTAL SECTION**

**Radiosynthesis of ¹⁸F-YJH08.** To identify optimal conditions for radiofluorination, the following workflow was applied by hand. An aqueous solution of H¹⁸F (20 mCi) was passed through a Sep-Pak light QMA cartridge, which was previously activated with distilled water (3 mL). A ¹⁸F fluoride ion was eluted with 0.5 mL of a K₂CO₃/KOTf solution (0.1 mg mL⁻¹ and 6 mg mL⁻¹ in 1:1 CH₃CN/H₂O). The solution was dried at 110 °C under a gentle stream of argon gas and then azeotropically dried twice with anhydrous acetonitrile (1 mL) at 110 °C. The mixture was heated to 110 °C for 30 min to remove the Boc protecting group. After the reaction was carefully monitored by radio-TLC for conversion.

On an ELIXYS FLEX/CHEM synthesis module (Sofi Biosciences), ¹⁸F-Boc-YJH08 was prepared in 90 min and then transferred from the ELIXYS on a C18 Sep-Pak cartridge. The ¹⁸F-Boc-YJH08 was eluted from the cartridge using anhydrous CH₃CN and purified by semipreparative HPLC (50% to 95% CH₃CN in water over 12 min and 95% CH₃CN in water for another 8 min) to obtain the desired compound in a decay-corrected radiochemical yield of 12.4 ± 3% (n = 10). The purity of the compound was further verified by reinjection on the semipreparative HPLC.

¹⁸F-YJH08 was dissolved in a 1.0 mL solution of 50% trifluoroacetic acid in CH₃CN, and the reaction was stirred at 40 °C for 30 min to remove the Boc protecting group. After the reaction was complete on TLC, the reaction vial was then cooled to 0 °C and quenched by adding DMF (0.5 mL), followed by the dropwise addition of 5 M NaOH solution (aq, 0.5 mL). The DCM was removed under reduced pressure, and the mixture was subjected to the semipreparative HPLC (30% to 95% CH₃CN in water over 12 min, then 95% CH₃CN for 8 min) for the purification, which provided the radioactive production in a decay-corrected radiochemical yield of 6.7 ± 1% (n = 10). The purity of the compound was further verified by reinjection of pure compound on semipreparative HPLC.

To prepare the radiotracer for animal studies, ¹⁸F-YJH08 was trapped on the cartridge and eluted with CH₃CN (1 mL). After removing the organic solvent, the pure product was dissolved in an injection formulation of 1/1/8 (v/v/v) DMSO/Tween 80/Saline. The stability, UV purity, and radiochemical purity of ¹⁸F-YJH08 in formulation was further confirmed by analytical HPLC equipped with a gamma ray radio detector and a UV detector at 254 nm. The analytical HPLC condition was as follows: Waters C18 (4.6 mm × 150 mm, 5 μm), mobile phase acetonitrile/H₂O, 8:2 (v/v); flow rate = 1 mL/min. Both the UV and Rad spectrum conformed with the analytical standard, and the purity of the tracer was ~97%.

**Animal Studies.** All animal experiments were conducted under the approval of the Institutional Animal Care and Use Committee (IACUC) at UCSF. Male nu/nu or C57BL6/J mice (4–6 weeks) were purchased from Charles River. All the mice were housed in a...
dedicated vivarium with free access to food and water. For dexamethasone treatment studies, mice were treated via oral gavage with vehicle (0.5% hydroxy-propyl-methylcellulose and 0.2% Tween 80 in water) or dexamethasone (100 mg/kg or 50 mg/kg). For JG231 treatment studies, mice were treated via intraperitoneal injection of JG231 (4 mg/kg) in injectable formulation (10% DMSO, 18% Tween80, 3.6% dextrose, 68.4% 1 M HEPES) or vehicle. The mice were treated once daily for 3 days. At day 3, the mice received the radiotracer after the final gavage or injection. Adrenalectomized mice were purchased from Charles River and provided drinking water supplemented with NaCl (aq) per instructions until the time of the tracer biodistribution study.

Small Animal PET/CT. Prior to injection, the formulated dose of $^{18}$F-YJH08 was passed through a sterile 0.22 μm membrane. Approximately 300 μCi was injected via tail vein in a volume of 100–150 μL per mouse. After the indicated period of uptake time, the mice were anesthetized with 2–3% isoflurane and imaged with a Siemens Inveon micro PET/CT. For dynamic acquisitions, the mice were anesthetized prior to injection and injected while positioned on the scanner bed. All imaging data were decay corrected, reconstructed, and analyzed with AMIDE software. Maximum intensity projections (MIPs) were generated by AMIDE software. Regions of interest (ROIs) were manually placed to calculate $SUV_{mean}$ data from the dynamic acquisitions.

Biodistribution Studies. Mice were euthanized after radiotracer injection with CO$_2$ (g) asphyxiation and dissected at dedicated time points post injection. The blood and tissues were removed, washed, dried, and weighed. The activity of each tissue was measured with a gamma counter. All data was decay corrected. PRISM software was used to express a percentage (% ID/g) of the injected dose per gram of tissue.

 Autoradiography. Twenty minutes postinjection of $^{18}$F-YJH08, mice were anesthetized and were perfused with cold PBS via cardiac puncture. Tissues were immediately collected and flash frozen in OCT on dry ice. Tissues were sectioned on a microtome at a thickness of 20 μm and immediately mounted on glass slides. The slides were then exposed on a GE phosphor screen storage, and the screen was developed on an Amersham Typhoon 9400 phosphorimager. H&E staining was performed by the Pathology Core Facility at UCSF. The autoradiography images were processed using ImageJ software.

Generation of the Transgenic Mice with Adipocyte Specific GR Knockout. To generate mice with Nr3c1 selectively deleted in mature adipocytes, female GR floxed mice (Jackson Laboratory, cat. no. 021021) were crossed to a male mouse carrying a transgene that expresses the Cre recombinase enzyme (Cre) under the regulation of the adiponectin promoter and enhancer elements (Jackson Laboratory, cat. no. 028020). This adiponectin-Cre (adipo-Cre) mouse line has expressed the Cre recombinase enzyme (Cre) under the regulation of mature adipocytes, female GR Knockout.

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Statistics. All statistical analysis was performed using PRISM v6.0 software. An unpaired, two-tailed Student’s t test was used to determine statistically significant differences in the data. Changes at the 95% confidence level ($P < 0.05$) were reported as statistically significant. For the determination of $K_r$ the data were fit with a one site nonlinear regression model.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschembio.9b01043.

Additional experimental details, figures, and schemes (PDF)

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