2-Amino-1,3-thiazol-4(5*H*)-ones as Potent and Selective 11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitors: Enzyme−Ligand Co-Crystal Structure and Demonstration of Pharmacodynamic Effects in C57Bl/6 Mice

Lars Johansson,*[,†] Christopher Fotsch,[‡] Michael D. Bartberger,[‡] Victor M. Castro,[†] Michelle Chen,[‡] Maurice Emery,[‡] Sonja Gustafsson,[†] Clarence Hale,[‡] Dean Hickman,[‡] Evert Homan,[†] Steven R. Jordan,[‡] Renee Komorowski,[‡] Aiwen Li,[‡] Kenneth McRae,[‡] George Moniz,[‡] Guy Matsumoto,[‡] Carlos Orihuela,[‡] Gunnar Palm,[†] Murielle Veniant,[‡] Minghan Wang,[‡] Meredith Williams,[†,§] and Jiandong Zhang[‡]

Biovitrum AB, SE-112 76 Stockholm, Sweden, and Amgen, Inc., One Amgen Center Drive, Thousand Oaks, California 91320

Received December 12, 2007

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) has attracted considerable attention during the past few years as a potential target for the treatment of diseases associated with metabolic syndrome. In our ongoing work on 11β-HSD1 inhibitors, a series of new 2-amino-1,3-thiazol-4(5*H*)-ones were explored. By inserting various cycloalkylamines at the 2-position and alkyl groups or spirocycloalkyl groups at the 5-position of the thiazolone, several potent 11β-HSD1 inhibitors were identified. An X-ray cocrystal structure of human 11β-HSD1 with compound **6d** (K_i = 28 nM) revealed a large lipophilic pocket accessible by substitution off the 2-position of the thiazolone. To increase potency, analogues were prepared with larger lipophilic groups at this position. One of these compounds, the 3-noradamantyl analogue **8b**, was a potent inhibitor of human 11β-HSD1 (K_i = 3 nM) and also inhibited 11β-HSD1 activity in lean C57Bl/6 mice when evaluated in an ex vivo adipose and liver cortisone to cortisol conversion assay.

Introduction

Type 2 diabetes (T2D)[a] constitutes a rapidly growing worldwide health problem, and it has been estimated that over 200 million people might be affected by the disease in 2010.[1] The pathogenesis of T2D is characterized by high plasma glucose levels, peripheral insulin resistance, and hyperinsulinemia.[2–4] At present, there are a number of drugs on the market that partially lower glucose levels and improve insulin sensitivity; however, more efficient therapies with less side effects are needed.[5] In this respect, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a biological target that has attracted considerable attention over the past few years.[6–8] This microsomal membrane-bound enzyme interconverts inactive cortisone (**1a**) to the receptor-active glucocorticoid cortisol (**2a**) in humans. In rodents, 11β-HSD1 catalyzes the corresponding conversion between 11-dehydrocorticosterone (**1b**) and corticosterone (**2b**) (Figure 1).[9,10]

Glucocorticoid hormones, such as cortisol, are important regulators of several intracellular transcription and expression processes. On the basis of observations from animal experiments, as well as studies with humans, it has become evident that glucocorticoid excess in tissues such as liver, adipose, and skeletal muscle might be contributing to the onset of the metabolic syndrome.[11,12] In the liver, gluconeogenesis, which is abnormally high in subjects with T2D,[13] is stimulated by cortisol via the nuclear glucocorticoid receptor (GR), primarily by activating the enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase.[14] It has been demonstrated that liver-specific GR antagonists suppress glucose output from the liver.[15] In the adipose tissue, cortisol is involved in the promotion of adipogenesis, induction of lipolysis, and release of free fatty acids.[16] The significance of glucocorticoids and 11β-HSD1 in T2D has been demonstrated in several rodent studies. 11β-HSD1 knockout mice showed resistance toward the development of diet-induced metabolic syndrome and had significantly lower fasting blood glucose levels as compared to wild-type mice.[17] In contrast, transgenic mice specifically overexpressing 11β-HSD1 in adipose tissue showed typical features of the metabolic syndrome such as visceral obesity, glucose intolerance, and insulin resistance.[18,19] Consistent with these findings, treatment of diabetic and obese mice with selective 11β-HSD1 inhibitors gave significantly improved insulin sensitivity and glucose tolerance and also reduced visceral fat deposits.[20,21] Moreover, clinical evidence in humans has been indirectly obtained with subjects suffering from Cushing's syndrome, where abnormal cortisol excess is characterized by impaired glucose tolerance, visceral obesity, and hyperglycaemia.[22] These findings suggest that inhibition of 11β-HSD1 might provide beneficial effects against diseases associated with metabolic syndrome.[23,24]

There are two known isoforms of 11β-HSD. The type 1 isoform (11β-HSD1) is primarily expressed in the liver and

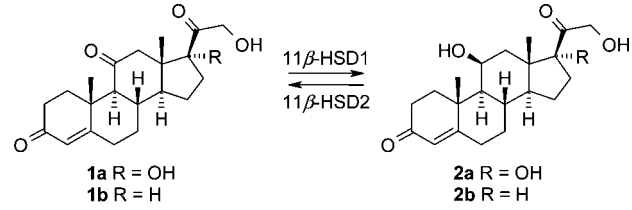


Figure 1. Interconversion of cortisone (**1a**)/11-dehydrocorticosterone (**1b**) and cortisol (**2a**)/corticosterone (**2b**).

* To whom correspondence should be addressed. Phone: +46-(0)8-6973804. Fax: +46-(0)8-6972320. E-mail: lars.johansson@biovitrum.com.
† Biovitrum AB.
‡ Amgen, Inc.
§ Current address: S*Bio Pte Ltd, 1 Science Park Road, no. 05-09 The Capricorn, Singapore Science Park II, Singapore 117528.

[a] Abbreviations: T2D, type 2 diabetes; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; GR, glucocorticoid receptor; 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; MR, mineralocorticoid receptor; SAR, structure−activity relationship; SPA, scintillation proximity assay; PK, pharmacokinetics; PD, pharmacodynamics; HTS, high-throughput screen; DPPA, diphenyl phosphorazidate; GA, 18β-glycyrrhetinic acid; CMC, carboxymethylcellulose.

10.1021/jm701551j CCC: $40.75
Published on Web 04/18/2008

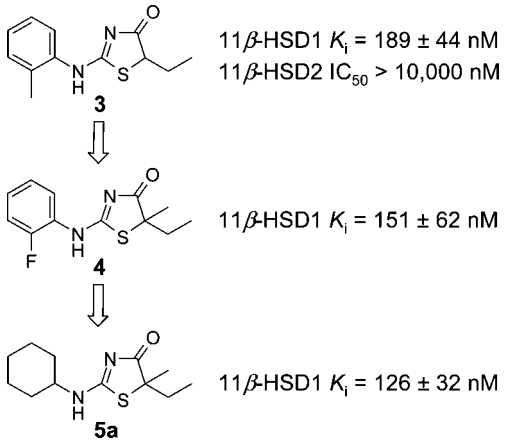


Figure 2. Progression of screening hit (**3**) to lead (**5a**). 11β-HSD1 potencies are expressed as the mean ± SD of at least three independent determinations.

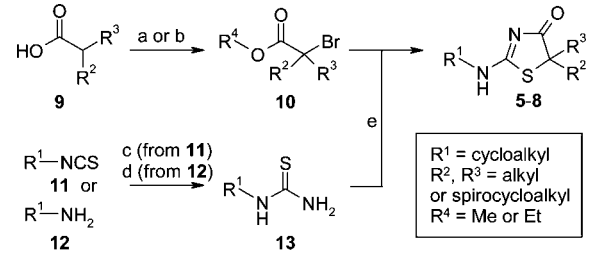
adipose tissue, whereas the type 2 isoform (11β-HSD2) is located mainly in the kidney. 11β-HSD1 is a NADPH-dependent, bidirectional enzyme, functioning predominantly as an oxoreductase producing active glucocorticoids. 11β-HSD2, on the other hand, is a NAD-dependent unidirectional enzyme that acts as a dehydrogenase to form inactive 11-keto metabolites and protects the mineralocorticoid receptor (MR) from cortisol. Inappropriate cortisol-induced activation of the MR might lead to sodium retention, hypokalemia, and hypertension,[25] so 11β-HSD2 inhibition should be avoided for any potential drug aimed at inhibiting 11β-HSD1.

In contributions from our laboratories[26] and others,[27–33] the discovery of selective, nonsteroidal, small-molecule 11β-HSD1 inhibitors has been reported during the past few years. As part of our ongoing efforts to develop 11β-HSD1 inhibitors for the treatment of T2D, the 1,3-thiazol-4(5*H*)-one (henceforth abbreviated as thiazolone) compound **3** was identified as a hit in a high-throughput screen of our compound collection (Figure 2). The compound was a moderate 11β-HSD1 inhibitor (K_i = 189 nM), selective versus 11β-HSD2, possessed good aqueous solubility, and had a relatively low molecular weight. Further optimization of this compound eventually led to compound **5a** (K_i = 126 nM), which no longer contained the aniline functionality at the 2-position (Figure 2). In this paper, we describe the structure−activity relationships (SAR) of a series of analogues to **5a** that led to the identification of a potent inhibitor of 11β-HSD1 with in vivo activity.

Chemistry

Synthesis. The synthetic routes toward the 5,5-dialkyl and 5-spirocycloalkyl substituted thiazolone end products **5**−**8** are outlined in Scheme 1. The key step in the synthesis, the construction of the thiazolone ring system, was accomplished by a substitution/cyclization reaction between a *N*-cycloalkylthiourea (**13**) and an α-bromoester (**10**).[34] The rate of this reaction step was found to be sensitive to the steric properties of the α-bromoesters **10**, and the process required long reaction times (3−8 days) by conventional heating at 90−105 °C. However, the reaction time could be substantially reduced by microwave-assisted heating at 155−170 °C in the presence of *N*,*N*′-diisopropylethylamine, and under these conditions the desired 5,5-dialkyl and 5-spirocycloalkyl analogues were obtained within 2.5 h. The noncommercially available α-bromoesters were either synthesized by (a) converting carboxylic acid (**9**) to the corresponding acyl chloride with thionyl chloride

Scheme 1[a]



[a] Reagents and conditions: (a) (i) $SOCl_2$, 60 °C, (ii) Br_2, HBr, 60−70 °C, (iii) MeOH, HCl, rt; (b) (i) PCl_3, Br_2, 85 °C, (ii) oxalyl chloride, DMF, CH_2Cl_2, rt, (iii) EtOH, $EtN(^iPr)_2$, rt; (c) NH_3, dioxane, rt; (d) (i) ethoxycarbonyl isothiocyanate, CH_2Cl_2, rt or benzoyl isothiocyanate, $CHCl_3$, rt, (ii) basic hydrolysis; (e) dioxane, 90−105 °C or $EtN(^iPr)_2$, ethanol, and microwave heating (155−170 °C).

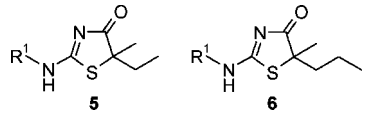
followed by mono α-bromination with Br_2 and catalytic HBr and subsequent reaction of the obtained α-brominated acyl chloride with methanol to give the desired α-bromoester (**10**), or (b) direct α-bromination of the carboxylic acid (**9**) using the Hell−Volhard−Zelinsky reaction,[35] followed by acyl chloride formation with oxalyl chloride and subsequent ester preparation to **10**. The cycloalkylthioureas **13** were prepared from the corresponding cycloalkylisothiocyanate (**11**) by addition of ammonia.[36] Alternatively, the synthesis of **13** was carried out by reacting the corresponding cycloalkylamine **12** with ethoxycarbonyl isothiocyanate or benzoyl isothiocyanate, followed by basic hydrolysis.[37] The noncommercially available cycloalkylamines **12** were prepared according to methodologies described in the literature.[38]

Results and Discussion

We initially focused our efforts on probing the importance of the size of the group at the thiazolone 2-position (Table 1, compounds **5a**−**d**). The inhibitory properties of the molecules were evaluated in a scintillation proximity assay (SPA) with human 11β-HSD1, and selected compounds were counter-screened in a human 11β-HSD2 assay. Potency in the 11β-HSD1 enzymatic binding assay improved about 2-fold, when compared to **5a**, by either extending the cyclohexyl ring at the 2-position by one methylene (**5b**) or by increasing the ring size (**5c** and **5d**). The enzymatic potency could be improved even further by substituting an *n*-propyl group for the ethyl group at the 5-position (**6a**−**d**). The most potent compound from this series, the cyclooctyl analogue **6d**, was a selective inhibitor for 11β-HSD1 with a K_i value of 28 nM and exhibited no appreciable activity against 11β-HSD2.

Compound **6d** was cocrystallized with human 11β-HSD1, and the X-ray structure was determined (Figure 3) (PDB ID for X-ray cocrystal structure of human 11β-HSD1 with **6d**: 3BYZ). The overall fold of the 11β-HSD1 protein in the structure is very similar to those previously published.[39–41] Even though racemic **6d** was used for the cocrystallization with human 11β-HSD1, only the C-5 (*S*)-stereoisomer ((*S*)-**6d**) cocrystallized with the enzyme. The inhibitor occupies the substrate binding site and thus competes with the substrate steroid. However, the aminothiazolone core of (*S*)-**6d** is in a reversed binding mode compared to our previously published structure of the *N*-(2-fluorophenyl) derivative **14**.[26e] Furthermore, it can be inferred from the hydrogen bonding pattern of the active site residues that (*S*)-**6d** exists as the *endo*-double bond tautomer, which is different from compound **14** where the bound tautomer contains the *exo*-double bond. In the cocrystal structure of **14**,[26e] the backbone NH proton of Ala172 forms a hydrogen bond with

Table 1. Human 11β-HSD1 and 11β-HSD2 Activities of Compounds **5** and **6**



5 **6**

R^1	compd[a]	11β-HSD1 K_i[b] (nM)	11β-HSD2 IC_{50}[c] (nM)
	**5a**	126 ± 32	nd
	6a	55 ± 20	nd
	**5b**	64 ± 14	nd
	6b	34 ± 9	nd
	**5c**	82 ± 29	>10,000
	6c	32 ± 9	>10,000
	**5d**	47 ± 26	>10,000
	6d	28 ± 9	>10,000
	**5e**	58 ± 24	nd
	6e	38 ± 16	nd
	**5f**	73 ± 37	>10,000
	6f	25 ± 10	nd
	**5g**	372 ± 79	nd
	6g	149 ± 76	>10,000
	**5h**	13 ± 8	>10,000
	6h	12 ± 1	nd
	**5i**	24 ± 5	nd
	6i	24 ± 11	nd

[a] Racemic mixtures. [b] Potencies are expressed as the mean ± SD of at least three independent determinations. [c] Single-point determinations at 10 μM concentration.

the oxygen lone pair supplied by the nearby side chain oxygen of Ser170 and the OH proton of Ser170 interacts with the acceptor lone pair on the exocyclic nitrogen of the inhibitor. In the cocrystal structure of (*S*)-**6d**, the backbone NH proton of Ala172 is in direct contact with the thiazolone carbonyl oxygen atom, and the side chain OH proton of Ser170 hydrogen bonds with the side chain oxygen of Tyr183. The NH protons on both inhibitors, exocyclic NH for analogue (*S*)-**6d** and endocyclic NH for analogue **14**, form hydrogen bonds with the side chain oxygen of Tyr183. The OH proton of Tyr183 in each cocrystal structure interacts with a ribose OH group of the adjacent NADPH cofactor, which in turn appears to be exposed to bulk solvent.

According to the X-ray structure with compound **14**, Tyr177 forms an edge-to-face aromatic stacking interaction with the 2-fluorophenyl moiety.[26e] In the X-ray with (*S*)-**6d**, Tyr177 adopted a change in conformation, accompanied by a rotation of the Cβ-Cγ bond of nearby residue Leu171, to form a van der Waals contact with the C-5 propyl substituent of (*S*)-**6d**. The C-5 methyl was only 3.6 Å from the backbone NH of Leu217. The proximity of the C-5 methyl group to the protein might explain why (*R*)-**6d** does not cocrystallize with human 11β-HSD1 because the propyl group on (*R*)-**6d** would sterically clash with Leu217. From analysis of the distances between the various methylene groups of the *N*-cyclooctyl function of (*S*)-**6d** to the cofactor and nearby residues in the active site (not shown), it was envisaged that even larger groups might be accommodated in the hydrophobic pocket occupied by the cyclooctyl group.

Armed with this structural information, we prepared analogues with bulkier cycloalkyl substituents at the 2-position of the thiazolone ring (Table 1, compounds **5e**−**i** and **6e**−**i**). Three of the compounds from this set, the 1-norbornyl analogue **6f** and the 1-adamantyl analogues **5i** and **6i**, showed comparable potency to the cyclooctyl analogue **6d**. However, the most potent compounds in the series were the 3-noradamantyl species **5h** and **6h** with 11β-HSD1 K_i values of 13 and 12 nM, respectively. Noteworthy is also that the 7-norbornyl analogues **5g** and **6g** were significantly less potent when compared to the corresponding 1-norbornyl species **5f** and **6f**. These results indicate that both the size and the shape of the cycloalkyl moiety in the thiazolone 2-position are of importance when optimizing the interactions in this hydrophobic region of the enzyme.

To exploit the second hydrophobic pocket close to the 5-position of the thiazolone, the diethyl analogues **7** and the spirocyclopentyl analogues **8** were prepared (Table 2). These structural changes not only increased the steric bulk at the thiazolone 5-position when compared to compounds **5** and **6** but they also had the advantage of giving achiral molecules. Of the six analogues prepared, the cycloctyl substituted thiazolone **8a** and the 3-noradamantyl analogue **8b**, both with the spirocyclopentyl group on the 5-position, showed improved potency over their 5-methyl-5-propyl congeners **6d** and **6h**. The most potent of these, **8b**, had an 11β-HSD1 K_i value of 3 nM, exhibited high selectivity versus 11β-HSD2, and was selected for further in vitro and in vivo profiling.

Before assessing the potency of compound **8b** in our rodent pharmacodynamic (PD) model, the binding affinity in the murine 11β-HSD1 assays and rat pharmacokinetics of **8b** were evaluated. As seen in Table 3, compound **8b** was 40−60-fold less potent against murine 11β-HSD1 when compared to human 11β-HSD1. This result is consistent with our previous observation that compounds that are active in the human 11β-HSD1 assay are not necessarily potent in the murine 11β-HSD1 assays. Such discrepancies in 11β-HSD1 affinity between species might be anticipated, e.g., the mouse and human 11β-HSD1 enzymes only share a 79% amino acid identity.[26a] Nonetheless, we deemed compound **8b** sufficiently potent against murine 11β-HSD1 to further investigate its pharmacokinetic profile in rats (Table 3). While the intravenous clearance was high (6.25 L h^{-1} kg^{-1}), the oral bioavailability and half-life (45% and 2 h, respectively) were both considered acceptable for an in vivo evaluation of **8b**.

In our rodent PD model, the inhibition of 11β-HSD1 was measured in adipose and liver, the two tissues where 11β-HSD1 is believed to play a role in metabolic diseases. Compound **8b** was administered to lean C57Bl/6 mice in a single 30 mg/kg oral dose, and after 2 and 6 h, the inhibition of 11β-HSD1 was measured ex vivo by incubating epididymal fat and liver in media containing ^{3}H-cortisone. Enzyme activity of 11β-HSD1 was significantly lower when compared to vehicle controls after 2 h (86% and 89% reduction in adipose and liver, respectively) and 6 h (87% and 79% reduction in adipose and liver, respectively) (Figure 4). To achieve this level of inhibition, the plasma concentrations of compound **8b** were 20 ± 1 μM at 2 h and 7.3 ± 0.5 μM at 6 h, which, after accounting for protein binding (mouse f_u = 0.63%), corresponds to fraction unbound concentrations of 120 and 44 nM, respectively. Considering the

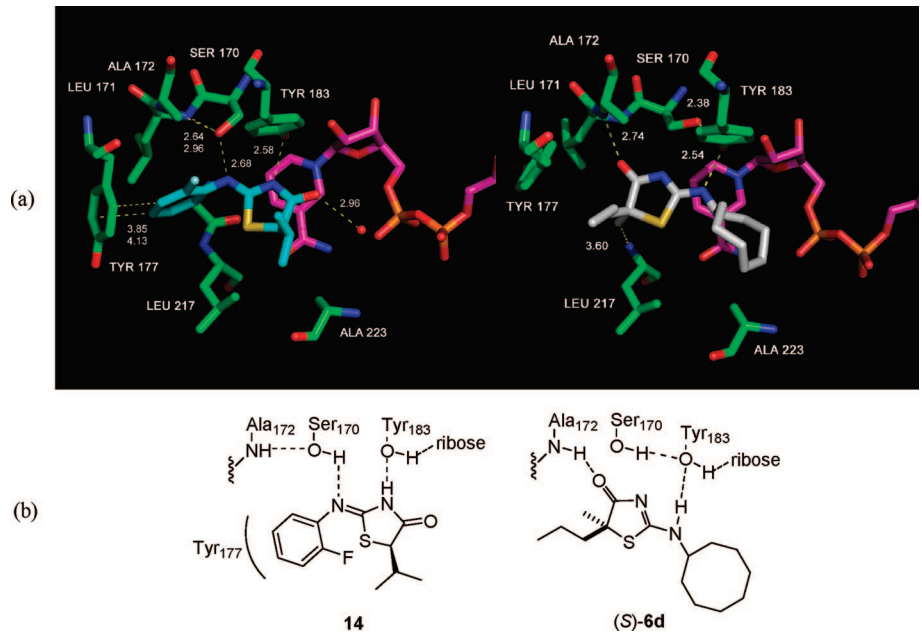
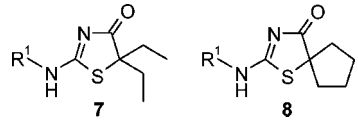
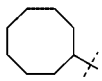
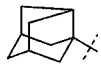
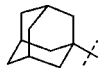


Figure 3. (a) Cocrystal structures of previously reported *N*-(2-fluorophenyl) analogue **14** (left) and *N*-cyclooctyl inhibitor (*S*)-**6d** (right) in the active site of human 11β-HSD1, depicting the reversal of binding mode of the thiazolone core (red = oxygen, blue = nitrogen, yellow = sulfur, orange = phosphorus, carbon: green = human 11β-HSD1, pink = NADPH, light blue = **14**, grey = (*S*)-**6d**). Distances are in Å; (b) schematic picture of the key interactions of **14** and (*S*)-**6d** with human 11β-HSD1.

Table 2. Human 11β-HSD1 and 11β-HSD2 Activities of Compounds **7** and **8**



R^1	compd	11β-HSD1 K_i[a] (nM)	11β-HSD2 IC_{50}[b] (nM)
	**7a**	26 ± 6	>10,000
	8a	14 ± 3	>10,000
	**7b**	13 ± 9	>10,000
	8b	3 ± 1	>10,000
	**7c**	20 ± 7	nd
	8c	28 ± 4	nd

[a] Potencies are expressed as the mean ± SD of at least three independent determinations. [b] Single-point determinations at 10 μM concentration.

moderate inhibitory potency against mouse 11β-HSD1 for **8b** (K_i = 120 nM), the great pharmacodynamic effects on 11β-HSD1 observed at these relatively low plasma concentrations might indicate that the distribution of **8b** into adipose and liver tissues is highly efficient. Furthermore, it cannot be excluded that hypothetical 11β-HSD1 active metabolites of **8b** are contributing to the observed inhibitory effects as well.

Conclusion

Optimization of the HTS hit compound **3** led to a series of potent and selective thiazolones with mono-, bi-, and tricycloalkylamines at the 2-position and short-chain alkyl groups or spirocycloalkyl groups at the 5-position. Many of the compounds showed low nanomolar potencies in the human 11β-HSD1 SPA binding assay and exhibited high selectivity for 11β-HSD1 versus 11β-HSD2. In the X-ray cocrystal structure of the cyclooctyl analogue (*S*)-**6d** with 11β-HSD1, we observed that the hydrophobic pockets near the catalytic site could accommodate larger groups, which might be exploited to improve potency. To that end, we prepared analogues with larger lipophilic groups on both sides of the thiazolone core and identified the achiral 3-noradamantyl analogue **8b**, which had an 11β-HSD1 K_i value of 3 nM. Compound **8b** was also orally active in mice, where it inhibited 11β-HSD1 activity in both the epididymal fat and liver. Our findings suggest that this type of thiazolones might provide suitable 11β-HSD1 inhibitors for further evaluation in preclinical models of diseases associated with metabolic syndrome.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on Varian Inova (400 MHz), Bruker DRX (500 MHz), Bruker 300-Avance (300 MHz), or on Avance DMX-400 (400 MHz) instruments. All spectra were recorded using the residual solvent proton resonance or tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in parts per million (ppm, δ units). Elemental analysis was performed by Atlantic Microlab, Inc., Norcross, GA. The purity of compounds was determined by analytical reverse-phase HPLC. The retention time (t_R) was determined on Agilent Series 1100 spectrometers using either ACE 3 C8 (3 μm, 3.0 mm × 50 mm) column (system A), Chromolith SpeedROD RP-18e (4.6 mm × 50 mm) column (system B), Phenomenex Synergi MAX-RP (2.0 mm × 50 mm) column (systems C and F), Phenomenex Luna C8(2) column (4.6 mm × 100 mm) (system D), or Agilent Zorbax SB-C18 (3.0 mm × 50 mm) column (system E). For systems A−C and F, 0.1% trifluoroacetic acid in acetonitrile (M1) and 0.1% trifluoroacetic acid in H_2O (M2) were used as mobile phases. Gradient system A: 0−3.0 min, 10−97% M1; 3.0−3.1 min, 97−100% M1; 3.1−4.1 min, 100% M1; 4.1−4.2 min, 100−10% M1; 4.2−6.2 min, 10% M1. Flow rate 0.7 mL/min. Injection volume 3.0 μL. Gradient system B: 0−2.0 min, 2−95% M1; 2.0−2.1 min, 95−100% M1; 2.1−3.0 min, 100% M1; 3.0−3.1 min, 100−2% M1; 3.1−3.6 min, 2% M1. Flow rate 5.0 mL/min. Injection volume 5.0 μL. Gradient system C: 0−0.2 min, 10% M1; 0.2−3.0 min, 10−100% M1; 3.0−4.5 min, 10% M1; 4.5−5.0 min, 100−10% M1. Flow rate 0.8 mL/min. Injection volume 4.0 μL.

Table 3. Mouse and Rat 11β-HSD1 Activities and Rat Pharmacokinetics of Compound **8b**

mouse 11β-HSD1 K_i[a] (nM)	rat 11β-HSD1 K_i[a] (nM)	CL[b] (L h^{-1} kg^{-1})	V_{ss}[b] (L kg^{-1})	t_{max}[c] (h)	$AUC_{0\text{-inf}}$[c] (ng h^{-1} mL^{-1})	$t_{1/2}$[c] (h)	$\%F_{oral}$[c]
120 ± 5	180 ± 33	6.25	1.84	2.5	175	2	45

[a] $n = 2$, mean ± SD reported. [b] Dosed iv (0.5 mg kg^{-1}) in DMSO. [c] Dosed po (2 mg kg^{-1} in 0.5% CMC/0.1% Tween 80 in water).

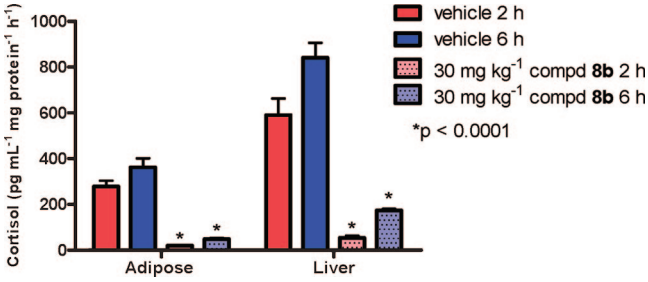


Figure 4. Ex vivo data for **8b**. Acute effects of **8b** as assessed by ex vivo cortisone to cortisol turnover. The compound was administered orally to lean C57Bl/6 mice (vehicle = 0.5% CMC/0.1% Tween 80 in water; $n = 6$/group). Animals were sacrificed at 2 and 6 h post dose, at which time inguinal fat pads and liver were removed to measure 11β-HSD1 activity. Values are expressed as mean ± SEM of $n = 6$.

Gradient system F: 0−0.2 min, 10% M1; 0.2−3.0 min, 10−95% M1; 3.5−3.7 min, 10% M1; 3.7−5.0 min, 100−10% M1. Flow rate 1.5 mL/min. Injection volume = 1.0 μL. For systems D and E, 0.1% formic acid in acetonitrile (M3) and 0.1% formic acid in H_2O (M4) were employed as mobile phases. Gradient system D: 0−0.5 min, 10% M3; 0.5−7.0 min, 10−100% M3; 7.0−9.5 min, 100% M3; 9.5−10.0 min, 100−10% M3. Flow rate 1.0 mL/min. Injection volume 3.0 μL. Gradient system E: 0−3.0 min, 10−95% M3; 3.0−3.5 min, 95% M3; 3.5−3.51 min, 95−10% M3. Flow rate 1.5 mL/min. Flow rate 1.5 mL/min. Injection volume 2.0 μL. Electrospray mass spectrometry (MS) was performed using an Agilent 1100 series liquid chromatograph/mass selective detector (MSD) to obtain the pseudomolecular $[M + H]^+$ ion of the target molecules. Accurate mass determinations were performed on the following two systems. (A) Agilent MSD-TOF (positive electrospray ionization) connected to an Agilent 1100 HPLC system with a diode array detector. The instrument was calibrated by Agilent ES-TOF tuning mix. (B) Bruker FTMS (positive electrospray ionization) with an external calibration using PEG 600 as the calibrant. Preparative HPLC was performed on a Gilson 305 HPLC system equipped with an ACE 5 C8 (5 μm, 21.2 mm × 100 mm) column, a Varian PrepStar HPLC system with a Phenomenex C18 (5 μm, 30 mm × 150 mm) column, or on a Waters 2700/Micromass ZQ system equipped with an ACE 5 C8 (5 μm, 21.2 mm × 100 mm) column. Acetonitrile and H_2O containing 0.1% TFA, respectively, were used as mobile phases. Preparative flash chromatography was carried out on Merck silica gel 60 (230−400 mesh) or prepacked silica gel cartridges (Biotage). Microwave reactions were performed with a Personal Chemistry Smith creator or synthesizer using 0.5−2 mL or 2−5 mL Smith process vials fitted with aluminum caps and septa. Unless otherwise noted, all materials were obtained from commercial sources and used without further purification. Norbornane-1-carboxylic acid was prepared from norbornane-2-carboxylic acid as described in the literature.[38] For a majority of the products **5**−**8**, ^{1}H NMR spectra with dual signal set were observed. The explanation to this feature is the existence of two rotameric forms due to restricted rotation around the thiazolone C2−NH bond.

Methyl 2-Bromo-2-methylbutanoate (10a). A solution of 2-methylbutanoic acid (3.00 g, 29.4 mmol) in thionyl chloride (20 mL) was stirred at 60 °C for 1 h. The solution was cooled to rt, and Br_2 (3.00 mL, 58.7 mmol, 2 equiv) and one drop of 48% aqueous HBr were added. The mixture was heated at 60 °C for 1 h and at 70 °C for 2 h. The solvent was removed, and the residual α-brominated acyl chloride intermediate was dissolved in methanol (40 mL) and 1.25 M HCl in methanol (1 mL) was added. The mixture was stirred at rt for 22 h. The solvent was removed, and to the residue was added diethyl ether (50 mL) and H_2O (25 mL). The phases were separated, and the organic phase was washed with brine (10 mL) and dried over $MgSO_4$. After filtration, the solvent was carefully removed to give **10a** as a colorless liquid (3.33 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.99 (t, $J = 7.3$ Hz, 3 H), 1.87 (s, 3 H), 2.14 (m, 2 H), 3.78 (s, 3 H).

Ethyl 2-Bromo-2-methylbutanoate (10b). This compound was prepared according to the method described for **10h**. A reaction between 2-methylbutyric acid (3.64 g, 35.6 mmol), bromine (2.10 mL, 41.0 mmol), phosphorus trichloride (0.15 mL, 1.78 mmol), and oxalyl chloride (3.50 mL, 38.9 mmol) gave **10b** as a colorless oil (4.90 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.99 (t, $J = 7.5$ Hz, 3 H), 1.31 (t, $J = 7.5$ Hz, 3 H), 1.88 (s, 3 H), 2.15 (q, $J = 8.0$ Hz, 2 H), 4.24 (q, $J = 7.0$ Hz, 2 H).

Methyl 2-Bromo-2-methylpentanoate (10c). This compound was prepared according to the method described for **10a**. Starting from 2-methylpentanoic acid (2.10 g, 18.1 mmol), the synthesis gave **10c** as a pale-yellow liquid (1.98 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (t, $J = 7.3$ Hz, 3 H), 1.26−1.52 (m, 2 H), 1.88 (s, 3 H), 2.08 (m, 2 H), 3.77 (s, 3 H).

Ethyl 2-Bromo-2-methylpentanoate (10d). This compound was prepared according to the method described for **10h**. A reaction between 2-methylvaleric acid (5.88 g, 50.6 mmol), bromine (3.00 mL, 58.2 mmol), phosphorus trichloride (0.22 mL, 2.5 mmol), and oxalyl chloride (4.73 mL, 53.1 mmol) gave **10d** as a pale oil (8.80 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (t, $J = 7.5$ Hz, 3 H), 1.13−1.60 (m, 5 H), 1.89 (s, 3 H), 2.11 (q, $J = 7.5$ Hz, 2 H), 4.25 (q, $J = 7.5$ Hz, 2 H).

Methyl 2-Bromo-2-ethylbutanoate (10e). This compound was prepared according to the method described for **10a**. Starting from 2-ethylbutanoic acid (3.00 g, 25.8 mmol), the synthesis gave **10e** as a colorless liquid (4.08 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.97 (t, $J = 7.3$ Hz, 6 H), 2.12 (m, 4 H), 3.77 (s, 3 H).

Ethyl 2-Bromo-2-ethylbutanoate (10f). This compound was prepared according to the method described for **10h**. A reaction between 2-ethylbutanoic acid (6.09 g, 51.9 mmol), bromine (3.10 mL, 60.0 mmol), phosphorus trichloride (0.23 mL, 2.6 mmol), and oxalyl chloride (4.90 mL, 54.6 mmol) gave **10f** as a pale oil (9.14 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.99 (t, $J = 7.2$ Hz, 6 H), 1.31 (t, $J = 7.2$ Hz, 3 H), 2.09−2.17 (m, 4 H), 4.25 (q, $J = 7.2$ Hz, 2 H).

Methyl 1-Bromocyclopentanecarboxylate (10g). This compound was prepared according to the method described for **10a**. Starting from cyclopentanecarboxylic acid (1.06 g, 9.26 mmol), the synthesis gave **10g** as a pale-yellow liquid (591 mg, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.77 (m, 2 H), 1.97 (m, 2 H), 2.29 (m, 4 H), 3.79 (s, 3 H).

Ethyl 1-Bromocyclopentanecarboxylate (10h). Phosphorus trichloride (0.54 mL, 6.2 mmol) was added dropwise to the mixture of cyclopentanecarboxylic acid (14.2 g, 124 mmol) and bromine (7.35 mL, 143 mmol). The reaction was then gradually heated to 85 °C and stirred at this temperature in a sealed vessel for 12 h. After cooling to rt, the mixture was partitioned between ethyl acetate and H_2O. The combined organic portions were washed with H_2O and brine and concentrated in vacuo to give 23.9 g of the α-brominated carboxylic acid intermediate. The intermediate (23.9 g, 124 mmol) was dissolved in CH_2Cl_2 (250 mL), mixed with oxalyl chloride (11.6 mL, 130 mmol), and two drops of DMF were added. After stirring at rt for 2 h, the solvent was removed in vacuo and then ethanol (50 mL) was added to the residue followed by the addition of *N,N*-diisopropylethylamine (22.7 mL, 130 mmol). The mixture was stirred at rt for 20 min. The solvent was removed in vacuo, and the residue was partitioned between diethyl ether and H_2O. The organic portion was washed with H_2O and brine and concentrated in vacuo. The crude product was filtered through a

plug of silica gel eluting with 10% ethyl acetate in hexanes. Removal of the solvent gave **10h** as a pale oil (21.2 g, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.31 (t, J = 7.1 Hz, 3 H), 1.70−1.87 (m, 2 H), 1.90−2.08 (m, 2 H), 2.28−2.35 (m, 4 H), 4.25 (q, J = 7.1 Hz, 2 H).

***N*-Cyclohexylthiourea (13a).** A mixture of isothiocyanatocyclohexane (6.74 g, 46.8 mmol) and 0.5 M ammonia in 1,4-dioxane (150 mL) in a 500 mL round-bottomed flask was stirred at rt under nitrogen overnight. The solvent was removed in vacuo to afford **13a** as a white solid (5.10 g, 69%). ^{1}H NMR (300 MHz, CD_3OD) δ: 1.12−1.45 (m, 5 H), 1.57−1.82 (m, 3 H), 1.83−2.00 (m, 2 H), 3.90−4.11 (m, 1 H).

***N*-(Cyclohexylmethyl)thiourea (13b).** This compound was prepared according to the method described for **13a**. A reaction between (isothiocyanatomethyl)cyclohexane (3.88 g, 25.0 mmol) and 0.5 M ammonia in 1,4-dioxane (100 mL) gave **13b** as a white solid (4.21 g, 98%). ^{1}H NMR (300 MHz, CD_3OD) δ: 0.80−1.07 (m, 2 H), 1.11−1.38 (m, 3 H), 1.47−1.83 (m, 6 H), 2.88−3.25(m, 2 H).

***N*\-Cycloheptylthiourea (13c).** This compound was prepared according to the method described for **13a**. A reaction between isothiocyanatocycloheptane (8.47 g, 54.6 mmol) and 0.5 M ammonia in 1,4-dioxane (220 mL) gave **13c** as a white solid (7.03 g, 75%). ^{1}H NMR (300 MHz, CD_3OD) δ: 1.40−1.80 (m, 10 H), 1.85−2.09 (m, 2 H), 4.15−4.35 (m, 1 H).

***N*-Cyclooctylthiourea (13d).** Benzoyl isothiocyanate (7.40 mL, 54.0 mmol) was added to a solution of cyclooctanamine (6.25 g, 49.1 mmol) in chloroform (200 mL) in a 500 mL round-bottomed flask containing a magnetic stirring bar. The mixture was stirred at rt overnight, after which the solvents were removed in vacuo. The residue was stirred with potassium carbonate (34.6 g, 250 mmol) in methanol (200 mL), H_2O (100 mL) and THF (100 mL) at rt overnight. The low boiling solvents were removed in vacuo, and the residue was partitioned between ethyl acetate and H_2O. The organic portion was washed with brine and concentrated in vacuo. The residue was purified by silica gel flash chromatography (0−100% of ethyl acetate in hexanes) to give **13d** as a white solid (8.50 g, 93%). ^{1}H NMR (300 MHz, CD_3OD) δ: 1.47−1.77 (m, 12 H), 1.78−1.94 (m, 2 H), 4.22−4.35 (m, 1 H).

1-(2,2,3,3-Tetramethylcyclopropyl)thiourea (13e). To a solution of 2,2,3,3-tetramethylcyclopropanecarboxylic acid (681 mg, 4.79 mmol) in dry acetonitrile (10 mL) was added triethylamine (668 μL, 4.79 mmol) and diphenyl phosphorazidate (DPPA) (1.03 mL, 4.79 mmol). The reaction mixture was stirred at 50 °C for 2 h and then cooled to rt. After addition of 1 M aqueous HCl (10 mL) the mixture was heated at reflux for 5 h. The organic solvent was then evaporated and the remaining aqueous solution was neutralized with solid $NaHCO_3$ and extracted with chloroform. Ethoxycarbonyl isothiocyanate (565 μL, 4.79 mmol) was added to the combined chloroform phases, and the reaction mixture was stirred at rt for 24 h. The solvent was evaporated in vacuo, and the crude material was purified by silica gel flash chromathography (cyclohexane/ethyl acetate, 95:5). The residue was suspended in 5 M aqueous NaOH (7 mL), and the reaction mixture was stirred at 50 °C for 3 h. The precipitate was collected by filtration, yielding 380 mg (46%) of **13e** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: ppm 1.03 (s, 6 H) 1.14 (s, 6 H) 1.91 (s, 1 H) 5.82 (s, 2 H) 6.12 (s, 1 H). MS (ESI+) for $C_8H_{16}N_2S$ *m*/*z*: 173 $[M + H]^+$.

***N*-(1-Norbornyl)thiourea (13f).** To a solution of norbornane-1-carboxylic acid (1.05 g, 7.48 mmol) and triethylamine (1.14 mL, 8.22 mmol) in dry acetonitrile (20 mL) was added DPPA (1.77 mL, 8.22 mmol), and the mixture was heated at 50 °C for 3 h. After cooling to rt, concentrated HCl (15 mL) was added and the mixture was heated under reflux (90 °C) for 3.5 h. The temperature was decreased to 85 °C, and the mixture was stirred for an additional 17 h. After cooling, solid $NaHCO_3$ was added until the pH was basic. H_2O and CH_2Cl_2 were added, and the mixture was vigorously stirred until everything dissolved. The phases were separated, and the aqueous layer was extracted with several portions of CH_2Cl_2 (10 × 100 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was evaporated to give the crude intermediate amine as a white solid (325 mg). The crude material was suspended in a mixture of CH_2Cl_2 (10 mL) and triethylamine (137 μL, 0.99 mmol), and after addition of ethoxycarbonyl isothiocyanate (117 μL, 0.99 mmol), the reaction was stirred at rt for 35 min. To the resulting pale-yellow solution was added 5 M NaOH (15 mL), and the reaction was heated at 50 °C for 3.5 h and at 65 °C for 1.5 h. The mixture was cooled, and CH_2Cl_2 (30 mL) was added. The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (20 mL), H_2O (15 mL), and brine (10 mL). Evaporation of the solvent and drying in vacuo yielded **13f** as an off-white solid (139 mg, 11%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.43−1.49 (m, 2 H), 1.64 (m, 2 H), 1.71−1.87 (m, 6 H), 2.24 (m, 1 H), 5.87 (br, 2 H), 6.70 (br, 1 H). MS (ESI+) for $C_8H_{14}N_2S$ *m*/*z*: 171 $[M + H]^+$.

***N*-(7-Norbornyl)thiourea (13g).** A 100 mL round-bottomed flask was charged with magnesium (639 mg, 26.3 mmol), and dry diethyl ether (6 mL) was added. A small portion of a solution of 7-bromonorbornane (4.18 g, 23.9 mmol) in dry diethyl ether (10 mL), and a few I_2 crystals were then added to the flask. The Grignard reaction was initiated by heating the mixture at reflux temperature under stirring. The remaining diethyl ether solution of 7-bromonorbornane was then added dropwise via a syringe over 10 min. The reaction was heated under reflux for a further 30 min and then cooled to rt. A rubber balloon was charged with CO_2(g), and via a syringe, the gas was slowly bubbled through the solution under stirring for 3.5 h. To the resulting suspension, diethyl ether (50 mL) and 2 M aqueous HCl (50 mL) were added. The phases were separated, and the aqueous layer was extracted with diethyl ether (2 × 50 mL). The combined organic phases were washed with brine (15 mL) and dried over $MgSO_4$. Removal of the solvent yielded 2.09 g of a white residue containing norbornane-7-carboxylic acid intermediate. To the crude material dissolved in acetonitrile (25 mL) was added triethylamine (2.27 mL, 16.4 mmol) and DPPA (3.54 mL, 16.4 mmol). After stirring the mixture at 50 °C for 2 h, *tert*-butanol (80 mL) was added and the reaction was heated at 80 °C overnight. The resulting mixture was concentrated under vacuum, and CH_2Cl_2 (100 mL) and saturated aqueous $NaHCO_3$ (50 mL) were added. The phases were separated, and the organic phase was washed with saturated aqueous $NaHCO_3$ (50 mL), H_2O (50 mL), brine (25 mL), and dried ($MgSO_4$). Evaporation of the solvent gave a sticky transparent solid that was dissolved in CH_2Cl_2 (10 mL). After addition of concentrated trifluoroacetic acid (5 mL), the solution was stirred under reflux overnight. The resulting solution was concentrated in vacuo to give the amine intermediate as an off-white sticky solid. The crude material was suspended in CH_2Cl_2 (10 mL) and triethylamine (2.3 mL), and after addition of ethoxycarbonyl isothiocyanate (1.94 mL, 16.4 mmol), the mixture was stirred at rt for 1 h. To the resulting solution was added 5 M NaOH (15 mL), and the reaction was heated at 65 °C for 3 h. The mixture was cooled, and CH_2Cl_2 (30 mL) was added. The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (20 mL), H_2O (15 mL), and brine (15 mL). Evaporation of the solvent and drying in vacuo gave a sticky solid residue. Further purification of the material by silica gel flash chromatography (pentane/ethyl acetate, 4:6) yielded the title compound **13g** as an off-white solid (382 mg, 15%, 78% pure by LCMS). MS (ESI+) for $C_8H_{14}N_2S$ *m*/*z*: 171 $[M + H]^+$. The material was used in subsequent reactions without any further purification.

***N*-(3-Noradamantyl)thiourea (13h).** To 3-noradamantyl amine (367 mg, 2.67 mmol) in CH_2Cl_2 (1 mL) was added dropwise ethoxycarbonyl isothiocyanate (316 μL, 2.67 mmol), and the mixture was stirred for 5 min. After addition of 5 M aqueous NaOH (8 mL), the reaction was stirred at 70 °C for 6 h. H_2O (20 mL) and ethyl acetate (25 mL) were added, and the layers were separated. The aqueous phase was extracted with ethyl acetate (5 mL), and the combined organic phases were dried over $MgSO_4$. After evaporation of the solvent and drying in vacuo, **13h** was obtained as a white solid (481 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ:

1.48−1.66 (m, 4 H), 1.81−1.96 (m, 4 H), 2.00−2.12 (m, 2 H), 2.33 (s, 2 H), 2.50 (m, 1 H), 6.04 (br, 2 H), 6.77 (br, 1 H).

***N*-(1-Adamantyl)thiourea (13i).** This compound was prepared according to the method described for **13a**. A reaction between 1-adamantyl isothiocyanate (4.83 g, 25.0 mmol) and 0.5 M ammonia in 1,4-dioxane (100 mL, 50 mmol) gave **13i** as a white solid (4.22 g, 80%). ^{1}H NMR (400 MHz, CD_3OD) δ: 1.70−1.75 (m, 6 H), 2.04−2.29 (m, 9 H).

(±)-2-(Cyclohexylamino)-5-ethyl-5-methyl-1,3-thiazol-4(5*H*)-one (5a). A 5 mL microwave vial containing a magnetic stirring bar was charged with *N*-cyclohexylthiourea (**13a**) (234 mg, 1.48 mmol), ethyl 2-bromo-2-methylbutanoate (**10b**) (310 mg, 1.48 mmol), *N*,*N*-diisopropylethylamine (0.30 mL), and ethanol (0.50 mL). The sealed vial was heated in a microwave oven at 155 °C for 2 h. After cooling to rt, the solid was collected by filtration, washed with ethanol, and dried under vacuum to give **5a** as a white solid (81 mg, 23%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.95 (t, J = 6 Hz, 3 H), 1.12−2.14 (m, 15 H), 3.16−4.19 (m, 1 H), 5.60 (br, 1 H, N*H*). Anal. ($C_{12}H_{20}N_2OS$) C, H, N.

(±)-2-(Cyclohexylamino)-5-methyl-5-propyl-1,3-thiazol-4(5*H*)-one (6a). A 5 mL microwave vial containing a magnetic stirring bar was charged with *N*-cyclohexylthiourea (**13a**) (340 mg, 2.15 mmol), ethyl 2-bromo-2-methylpentanoate (**10d**) (480 mg, 2.15 mmol), *N*,*N*-diisopropylethylamine (0.50 mL), and ethanol (1.0 mL). The sealed vial was heated in a microwave oven at 155 °C for 2 h, then at 170 °C for 30 min. After cooling to rt, the solvents were removed in vacuo. The residue was partitioned between ethyl acetate and H_2O. The combined organic portions were washed with brine and dried over Na_2SO_4. After removing the solvent, the residue was purified by preparative reverse-phase HPLC. The fractions containing the product were combined, and acetonitrile was removed in vacuo. The aqueous solution was then neutralized with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic portion was washed with brine and dried over Na_2SO_4. The solvent was evaporated to give **6a** as a tan solid (130 mg, 23%). ^{1}H NMR (400 MHz, $CDCl_3$ + 1 drop of TFA) δ: 0.99 (t, 3 H, J = 7.5 Hz), 1.24−1.37 (m, 4 H), 1.52−1.61 (m, 3 H), 1.68−1.71 (m, 1 H), 1.77 (s, 3H), 1.88−1.94 (m, 3 H), 1.98−2.05 (m, 3 H) 3.32 (m, 1 H). Anal. ($C_{13}H_{22}N_2OS$) C, H, N.

(±)-2-((Cyclohexylmethyl)amino)-5-ethyl-5-methyl-1,3-thiazol-4(5*H*)-one (5b). This compound was prepared according to the method described for **6a**. A reaction between *N*-(cyclohexylmethyl)thiourea (**13b**) (264 mg, 1.53 mmol) and ethyl 2-bromo-2-methylbutanoate (**10b**) (320 mg, 1.53 mmol) at 155 °C for 2 h gave **5b** as a white solid after extraction with CH_2Cl_2 (83 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$ + 1 drop of TFA) δ: 0.97−1.02 (m, 2 H), 1.05 (t, 3 H, J = 7.0 Hz), 1.14−1.34 (m, 3 H), 1.70−1.79 (m, 9 H), 1.90−1.99 (m, 1 H), 2.07−2.16 (m, 1 H), 3.32 (d, 2 H, J = 6 Hz). Anal. ($C_{13}H_{22}N_2OS\cdot0.1H_2O$) C, H, N.

(±)-2-((Cyclohexylmethyl)amino)-5-methyl-5-propyl-1,3-thiazol-4(5*H*)-one (6b). This compound was prepared according to the method described for **6a**. A reaction between *N*-(cyclohexylmethyl)thiourea (**13b**) (320 mg, 1.84 mmol) and ethyl 2-bromo-2-methylpentanoate (**10d**) (410 mg, 1.84 mmol) gave **6b** as a tan solid (82 mg, 17%). ^{1}H NMR (400 MHz, $CDCl_3$ + 1 drop of TFA) δ: 0.99 (t, 3 H, J = 7.5 Hz), 1.02−1.05 (m, 2 H), 1.13−1.36 (m, 4 H), 1.49−1.57 (m, 1 H), 1.70−1.79 (m, 9 H), 1.85−1.93 (m, 1 H), 1.98−2.05 (m, 1 H), 3.26 (d, 2 H, J = 6 Hz). Anal. ($C_{14}H_{24}N_2OS\cdot0.1H_2O$) C, H, N.

(±)-2-(Cycloheptylamino)-5-ethyl-5-methyl-1,3-thiazol-4(5*H*)-one (5c). A solution of *N*-cycloheptylthiourea (**13c**) (91.8 mg, 0.533 mmol) and methyl 2-bromo-2-methylbutanoate (**10a**) (104 mg, 0.533 mmol) in 1,4-dioxane (600 μL) was stirred at 95 °C in a sealed tube for 3 days. The solvent was removed, and the residue was purified by preparative reverse-phase HPLC. The fractions containing pure product were combined, and ethyl acetate (50 mL) and saturated aqueous $NaHCO_3$ (50 mL) were added. The phases were separated, and the aqueous layer was extracted with ethyl acetate (25 mL). The combined organic phases were washed with brine (25 mL) and dried over $MgSO_4$. Evaporation of the solvent, and drying in vacuo gave **5c** as a white solid (56 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.77−0.82 (m, 3 H), 1.34−1.64 (m, 13 H), 1.71 (q, J = 7.3 Hz, 2 H), 1.83−1.91 (m, 2 H), 3.35−3.96 (m, 1 H), 9.10−9.67 (m, 1 H, N*H*). HRMS m/z calcd for $C_{13}H_{22}N_2OS$: 254.1453; found: 254.1448. HPLC 100%, t_R = 2.81 min (system A), 100%, t_R = 1.30 min (system B).

(±)-2-(Cycloheptylamino)-5-methyl-5-propyl-1,3-thiazol-4(5*H*)-one (6c). This compound was prepared according to the method described for **5c**. A reaction between *N*-cycloheptylthiourea (**13c**) (76.5 mg, 0.444 mmol) and methyl 2-bromo-2-methylpentanoate (**10c**) (92.8 mg, 0.444 mmol) at 90 °C for 6 days gave **6c** as a white solid (47 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.82−0.86 (m, 3 H), 0.96−1.11 (m, 1 H), 1.33−1.73 (m, 16 H), 1.83−1.91 (m, 2 H), 3.36−3.96 (m, 1 H), 9.08−9.65 (m, 1 H, N*H*). HRMS m/z calcd for $C_{14}H_{24}N_2OS$: 268.1609; found: 268.1618. HPLC 100%, t_R = 3.00 min (system A), 100%, t_R = 1.43 min (system B).

(±)-2-(Cyclooctylamino)-5-ethyl-5-methyl-1,3-thiazol-4(5*H*)-one (5d). This compound was prepared according to the method described for **5c**. A reaction between *N*-cyclooctylthiourea (**13d**) (114 mg, 0.610 mmol) and methyl 2-bromo-2-methylbutanoate (**10a**) (119 mg, 0.610 mmol) at 95 °C for 5 days gave **5d** as a white solid (68 mg, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.77−0.86 (m, 3 H), 1.41−1.82 (m, 19 H), 3.42−4.01 (m, 1 H), 9.09−9.66 (m, 1 H, N*H*). HRMS m/z calcd for $C_{14}H_{24}N_2OS$: 268.1609; found: 268.1601. HPLC 100%, t_R = 3.01 min (system A), 100%, t_R = 1.41 min (system B).

(±)-2-(Cyclooctylamino)-5-methyl-5-propyl-1,3-thiazol-4(5*H*)-one (6d). This compound was prepared according to the method described for **5c**. A reaction between *N*-cyclooctylthiourea (**13d**) (82.7 mg, 0.444 mmol) and methyl 2-bromo-2-methylpentanoate (**10c**) (92.8 mg, 0.444 mmol) at 90 °C for 6 days gave **6d** as a white solid (57 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.82−0.86 (m, 3 H), 0.96−1.09 (m, 1 H), 1.31−1.81 (m, 20 H), 3.40−4.01 (m, 1 H), 9.07−9.65 (m, 1 H, N*H*). HRMS m/z calcd for $C_{15}H_{26}N_2OS$: 282.1766; found: 282.1774. HPLC 100%, t_R = 3.14 min (system A), 100%, t_R = 1.53 min (system B).

(±)-5-Ethyl-5-methyl-2-[(2,2,3,3-tetramethylcyclopropyl)amino]-1,3-thiazol-4(5*H*)-one (5e). This compound was prepared according to the method described for **5c**. A reaction between *N*-(2,2,3,3-tetramethylcyclopropyl)thiourea (**13e**) (70.4 mg, 0.409 mmol) and methyl 2-bromo-2-methylbutanoate (**10a**) (79.7 mg, 0.533 mmol) gave **5e** as a white solid (16 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.76−0.81 (m, 3 H), 0.92 (s, 1 H), 0.94 (s, 1 H), 0.96 (s, 2 H), 0.96 (s, 2 H), 1.06 (s, 1 H), 1.07 (s, 1 H), 1.08 (s, 2 H), 1.09 (s, 2 H), 1.46 (s, 1 H), 1.48 (s, 2 H), 1.69−1.78 (m, 2 H), 2.06−2.38 (m, 1 H), 8.81−9.68 (m, 1 H, N*H*). HRMS m/z calcd for $C_{13}H_{22}N_2OS$: 254.1453; found: 254.1443. HPLC 100%, t_R = 2.94 min (system A), 100%, t_R = 1.36 min (system B).

(±)-5-Methyl-5-propyl-2-[(2,2,3,3-tetramethylcyclopropyl)amino]-1,3-thiazol-4(5*H*)-one (6e). This compound was prepared according to the method described for **5c**. A reaction between *N*-(2,2,3,3-tetramethylcyclopropyl)thiourea (**13e**) (83.0 mg, 0.482 mmol) and methyl 2-bromo-2-methylpentanoate (**10c**) (101 mg, 0.482 mmol) at 90 °C for 6 days gave **6e** as a white solid (15 mg, 11%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.81−0.86 (m, 3 H), 0.92 (s, 1 H), 0.94 (s, 1 H), 0.95 (s, 2 H), 0.96 (s, 2 H), 0.98−1.06 (m, 1 H), 1.06 (s, 1 H), 1.06 (s, 1 H), 1.08 (s, 2 H), 1.09 (s, 2 H), 1.31−1.42 (m, 1 H), 1.45 (s, 1 H), 1.47 (s, 2 H), 1.61−1.77 (m, 2 H), 2.05−2.38 (m, 1 H), 8.80−9.61 (m, 1 H, N*H*). HRMS m/z calcd for $C_{14}H_{24}N_2OS$: 268.1609; found: 268.1609. HPLC 100%, t_R = 3.12 min (system A), 100%, t_R = 1.49 min (system B).

(±)-5-Ethyl-5-methyl-2-(1-norbornylamino)-1,3-thiazol-4(5*H*)-one (5f). This compound was prepared according to the method described for **5c**. A reaction between *N*-(1-norbornyl)thiourea (**13f**) (61.3 mg, 0.360 mmol) and methyl 2-bromo-2-methylbutanoate (**10a**) (70.2 mg, 0.360 mmol) at 95 °C for 3 days and 100 °C for 1 day yielded **5f** as a white solid (49 mg, 38%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.79 (t, J = 7.3 Hz, 3 H), 1.27−1.40 (m, 2 H), 1.45−1.47 (m, 3 H), 1.62−1.86 (m, 10 H), 2.11−2.16 (m, 1 H), 9.33−9.93 (m, 1 H, N*H*). HRMS m/z calcd for $C_{13}H_{20}N_2OS$:

252.1296; found: 252.1296. HPLC 100%, t_R = 2.84 min (system A), 96%, t_R = 1.22 min (system B).

(±)-5-Methyl-2-(1-norbornylamino)-5-propyl-1,3-thiazol-4(5*H*)-one (6f). This compound was prepared according to the method described for **5c**. A reaction between *N*-(1-norbornyl)thiourea (**13f**) (99.4 mg, 0.583 mmol) and methyl 2-bromo-2-methylpentanoate (**10c**) (122 mg, 0.583 mmol) at 110 °C for 4 days yielded **6f** as a white solid (42 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.84−0.96 (m, 3 H), 1.14−1.27 (m, 1 H), 1.33−1.50 (m, 3 H), 1.58−1.60 (m, 3 H), 1.66−2.03 (m, 10 H), 2.15−2.28 (m, 1 H), 6.52 and 10.11 (br, 1 H, N*H*). HRMS *m*/*z* calcd for $C_{14}H_{22}N_2OS$: 266.1453; found: 266.1445. HPLC 100%, t_R = 3.15 min (system A), 100%, t_R = 1.34 min (system B).

(±)-5-Ethyl-5-methyl-2-(7-norbornylamino)-1,3-thiazol-4(5*H*)-one (5g). This compound was prepared according to the method described for **5c**. A reaction between *N*-(7-norbornyl)thiourea (**13g**) (86.1 mg, 0.505 mmol) and methyl 2-bromo-2-methylbutanoate (**10a**) (98.6 mg, 0.505 mmol) at 100 °C for 4 days gave **5g** as a white solid (19 mg, 15%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91−0.95 (m, 3 H), 1.21−1.42 (m, 5 H), 1.60−1.98 (m, 9 H), 2.30−2.47 (m, 2 H), 3.46−4.12 (m, 1 H). HRMS *m*/*z* calcd for $C_{13}H_{20}N_2OS$: 252.1296; found: 252.1293. HPLC 97%, t_R = 2.80 min (system A), 93%, t_R = 1.22 min (system B).

(±)-5-Methyl-2-(7-norbornylamino)-5-propyl-1,3-thiazol-4(5*H*)-one (6g). This compound was prepared according to the method described for **5c**. A reaction between *N*-(7-norbornyl)thiourea (**13g**) (87.2 mg, 0.512 mmol) and methyl 2-bromo-2-methylpentanoate (**10c**) (107 mg, 0.512 mmol) at 100 °C for 4 days gave **6g** as a white solid (19 mg, 14%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.85−0.93 (m, 3 H), 1.16−1.91 (m, 15 H), 2.29−2.47 (m, 2 H), 3.44−4.12 (m, 1 H). HRMS *m*/*z* calcd for $C_{14}H_{22}N_2OS$: 266.1453; found: 266.1443. HPLC 100%, t_R = 2.96 min (system A), 100%, t_R = 1.35 min (system B).

(±)-5-Ethyl-5-methyl-2-(3-noradamantylamino)-1,3-thiazol-4(5*H*)-one (5h). This compound was prepared according to the method described for **5c**. A reaction between *N*-(3-noradamantyl)thiourea (**13h**) (103 mg, 0.522 mmol) and methyl 2-bromo-2-methylbutanoate (**10a**) (102 mg, 0.522 mmol) gave **5h** as a white solid (47 mg, 32%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.79 (t, J = 7.3 Hz, 3 H), 1.46 (s, 3 H), 1.47−1.57 (m, 4 H), 1.71 (q, J = 7.3 Hz, 2 H), 1.89−2.10 (m, 6 H), 2.21−2.29 (m, 2 H), 2.45 (m, 1 H), 9.23 (s, 1 H). HRMS *m*/*z* calcd for $C_{15}H_{22}N_2OS$: 278.1453; found: 278.1459. HPLC 100%, t_R = 2.98 min (system A), 100%, t_R = 1.36 min (system B).

(±)-5-Methyl-2-(3-noradamantylamino)-5-propyl-1,3-thiazol-4(5*H*)-one (6h). This compound was prepared according to the method described for **5c**. A reaction between *N*-(3-noradamantyl)thiourea (**13h**) (117 mg, 0.596 mmol) and methyl 2-bromo-2-methylpentanoate (**10c**) (125 mg, 0.596 mmol) at 95 °C for 4 days gave **6h** as a white solid (65 mg, 37%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.82−0.91 (m, 3 H), 1.03 (m, 1 H), 1.33−1.73 (m, 10 H), 1.88−2.10 (m, 6 H), 2.21−2.29 (m, 2 H), 2.45 (m, 1 H), 9.22−9.78 (m, 1 H, N*H*). HRMS *m*/*z* calcd for $C_{16}H_{24}N_2OS$: 292.1609; found: 292.1614. HPLC 92%, t_R = 3.16 min (system A), 97%, t_R = 1.49 min (system B).

(±)-2-(1-Adamantylamino)-5-ethyl-5-methyl-1,3-thiazol-4(5*H*)-one (5i). This compound was prepared according to the method described for **6a**. A reaction between *N*-(1-adamantyl)thiourea (**13i**) (270 mg, 1.29 mmol) and ethyl 2-bromo-2-methylbutanoate (**10b**) (270 mg, 1.29 mmol) at 155 °C for 2 h gave **5i** as a white solid (26 mg, 7%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90−1.02 (m, 3 H), 1.57−2.28 (m, 20 H), 5.45 (br, 1 H, N*H*). Anal. ($C_{16}H_{24}N_2OS\cdot0.5H_2O$) C, H, N.

(±)-2-(1-Adamantylamino)-5-methyl-5-propyl-1,3-thiazol-4(5*H*)-one (6i). This compound was prepared according to the method described for **6a**. A reaction between *N*-(1-adamantyl)thiourea (**13i**) (460 mg, 2.20 mmol) and ethyl 2-bromo-2-methylpentanoate (**10d**) (490 mg, 2.20 mmol) gave **6i** as a tan solid after extraction with CH_2Cl_2 (41 mg, 6%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90−1.00 (m, 3 H), 1.18−1.60 (m, 3 H), 1.61−2.20 (m, 19 H), 5.48 (br, 1 H, N*H*). Anal. ($C_{17}H_{26}N_2OS\cdot0.14CH_2Cl_2$) C, H, N.

2-(Cyclooctylamino)-5,5-diethyl-1,3-thiazol-4(5*H*)-one (7a). This compound was prepared according to the method described for **5c**. A reaction between *N*-cyclooctylthiourea (**13d**) (109 mg, 0.583 mmol) and methyl 2-bromo-2-ethylbutanoate (**10e**) (122 mg, 0.583 mmol) at 95 °C for 5 days and 105 °C for 3 days gave **7a** as a white solid (15 mg, 9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.85−0.95 (m, 6 H), 1.45−2.05 (m, 18 H), 3.55 and 4.28 (m, 1 H), 5.85 and 10.08 (m, 1 H, N*H*). HRMS *m*/*z* calcd for $C_{15}H_{26}N_2OS$: 282.1766; found: 282.1758. HPLC 98%, t_R = 3.11 min (system A), 100%, t_R = 1.52 min (system B).

2-(Cyclooctylamino)-1-thia-3-azaspiro[4.4]non-2-en-4-one (8a). This compound was prepared according to the method described for **6c**. A reaction between *N*-cyclooctylthiourea (**13d**) (77.3 mg, 0.415 mmol) and methyl 1-bromocyclopentanecarboxylate (**10g**) (85.9 mg, 0.415 mmol) at 90 °C for 3 days yielded **8a** as a white solid (24 mg, 20%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.41−1.70 (m, 20 H), 2.08−2.17 (m, 2 H), 4.04 (m, 1 H), 9.10 (d, J = 7.6 Hz, 1 H). HRMS *m*/*z* calcd for $C_{15}H_{24}N_2OS$: 280.1609; found: 280.1611. HPLC 99%, t_R = 3.11 min (system A), 100%, t_R = 1.46 min (system B).

5,5-Diethyl-2-(3-noradamantylamino)-1,3-thiazol-4(5*H*)-one (7b). This compound was prepared according to the method described for **5c**. A reaction between *N*-(3-noradamantyl)thiourea (**13h**) (115 mg, 0.588 mmol) and methyl 2-bromo-2-ethylbutanoate (**10e**) (123 mg, 0.588 mmol) at 95 °C for 5 days and 110 °C for 3 days gave **7b** as a white solid (12 mg, 7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.88−1.00 (m, 6 H), 1.45−2.35 (m, 16 H), 2.48 and 2.67 (m, 1 H), 6.08 and 8.96 (m, 1 H, N*H*). HRMS *m*/*z* calcd for $C_{16}H_{24}N_2OS$: 292.1609; found: 292.1622. HPLC 99%, t_R = 3.17 min (system A), 100%, t_R = 1.54 min (system B).

2-(3-Noradamantylamino)-1-thia-3-azaspiro[4.4]non-2-en-4-one (8b). This compound was prepared according to the method described for **5a**. A reaction between *N*-(3-noradamantyl)thiourea (**13h**) (320 mg, 1.63 mmol) and ethyl 1-bromocyclopentanecarboxylate (**10h**) (360 mg, 1.63 mmol) yielded **8b** as a white solid (165 mg, 35%). ^{1}H NMR (300 MHz, CD_3OD) δ: 1.52−2.39 (m, 20 H), 2.50−2.68 (m, 1 H). HRMS *m*/*z* calcd for $C_{16}H_{22}N_2OS$ [M + H]$^+$: 291.1526; found: 291.1533. HPLC 98%, t_R = 3.06 min (system C), 99%, t_R = 7.01 min (system D).

2-(1-Adamantylamino)-5,5-diethyl-1,3-thiazol-4(5*H*)-one (7c). This compound was prepared according to the method described for **6a**. A reaction between *N*-(1-adamantyl)thiourea (**13i**) (370 mg, 1.75 mmol) and ethyl 2-bromo-2-ethylbutanoate (**10f**) (399 mg, 1.75 mmol) gave **7c** as an off-white solid (55 mg, 10%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.73−0.85 (m, 6 H), 1.52−1.89 (m, 11 H), 1.98−2.11 (m, 8 H), 8.82 (br, 1 H, N*H*). HRMS *m*/*z* calcd for $C_{17}H_{26}N_2OS$ [M + H]$^+$: 307.1844; found: 307.1828. HPLC 100%, t_R = 2.92 min (system E), 100%, t_R = 7.38 min (system D).

2-(1-Adamantylamino)-1-thia-3-azaspiro[4.4]non-2-en-4-one (8c). This compound was prepared according to the method described for **6a**. A reaction between *N*-(1-adamantyl)thiourea (**13i**) (248 mg, 1.18 mmol) and ethyl 1-bromocyclopentanecarboxylate (**10h**) (260 mg, 1.18 mmol) at 170 °C for 1.5 h gave **8c** as a white solid (93 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.63−1.90 (m, 8 H), 1.96−2.27 (m, 13 H), 2.36−2.55 (m, 2 H), 5.38 (br, 1 H, N*H*). HRMS *m*/*z* calcd for $C_{17}H_{24}N_2OS$ [M + H]$^+$: 305.1682; found: 305.1684. HPLC 97%, t_R = 2.32 min (system F), 100%, t_R = 7.26 min (system D).

Biology. Materials. 1,2(*n*)-^{3}*H*]-Cortisone and [1,2,6,7-^{3}*H*]-hydrocortisone were purchased from Amersham. NADPH (tetrasodium salt) and NAD^+ (lithium salt, 10 mM stock in Tris buffer) were obtained from Sigma-Aldrich. Glucose-6-phosphate (G-6-P), 18β-glycyrrhetinic acid (GA), and carbenoxolone (disodium salt) were supplied by Sigma-Aldrich. Anticortisol monoclonal mouse antibody, clone 6D6.7, was obtained from Beckman Coulter (Marseille, France), and yttrium silicate (YSi) scintillation proximity assay (SPA) beads coated with monoclonal antimouse antibodies were from Amersham. The human 11β-HSD1 enzyme used was expressed in *Escherichia coli*.[42] The 11β-HSD2 enzyme was produced in HEK-293 cells transfected with a pcDNA3-derived plasmid. The 3-fold serial dilutions of test compounds in assay

buffer (30 mM Tris-HCl, pH 7.2 containing 1 mM EDTA) were performed on a Tecan Genesis RSP 150. The final concentration of the compounds spanned from 18 μM to 308 pM. Triplicates of the serial dilutions were performed on a Tomtec Quadra 96 instrument using 96-well microtiter plates (Perkin-Elmer, white Optiplate-96). The amount of the product [^{3}H]-cortisol bound to the beads was determined in a Packard Top Count NXT microplate liquid scintillation counter. Dilutions of stock solutions were made in assay buffer unless otherwise indicated.

Human 11β-HSD1 Scintillation Proximity Assay (SPA). The 11β-HSD1 enzyme assay was carried out in the replica plates of the compounds in a total well volume of 110 μL. Each reaction replica contained 10 μL of diluted compound, 50 μL of assay buffer, and 25 μL of substrate mixture [^{3}H]-cortisone/NADPH (175 nM/200 μM). Reactions were initiated by the addition of 25 μL of 11β-HSD1 enzyme (40−60 nM final concentration, depending on the batch). Following mixing, the plates were incubated on a shaker at rt for 30−60 min depending on batch. The reactions were terminated by addition of 10 μL of stop solution (1 mM GA in ethanol). Mouse monoclonal anticortisol antibody was then added (10 μL of 1.92 μM working solution) followed by 50 μL of YSi SPA beads (suspended according to the manufacturer instructions). Appropriate controls were set up, i.e., determinations of nonspecific binding (NSB) and of total activity (TOT), by adding or omitting 10 μL of 1 mM GA, respectively, before starting the reaction. As reference substance carbenoxolone was run in each plate. The plates were sealed with plastic film (Perkin-Elmer, Top Seal-A) and incubated on a shaker for 30 min at rt before counting. The amount of the product, [^{3}H]-cortisol, captured on the beads was determined in a microplate liquid scintillation counter. Kinetic constants were calculated employing the Microsoft Excel integrated application XLfit (Version 5.3.0.19, ID Business Solutions Ltd.) using the sigmoidal dose−response model 205, which is based on the nonlinear curve fitting based on Levenberg−Marquardts algorithm.

Human 11β-HSD2 Assay. The assay was performed at rt in a 96-well microtiter plate (Costar 96 well V-bottom polypropylene, catalogue no. 3363) containing 160 μL of substrate mixture [^{3}H]-cortisol/NAD$^+$ (200 nM/200 μM) in assay buffer, 20 μL of a 10 μM solution of inhibitors or control substance, and 20 μL of 11β-HSD2 (crude extract from sonicated HEK-293 cells diluted 10−20 fold in assay buffer). When a dose response was desirable, the inhibitor was diluted in a 3-fold manner from 10 μM to 169 pM. Pure DMSO was diluted in parallel with inhibitors and used to assess the solvent effect on the enzyme. Following incubation for 10 min, the reaction was terminated with perchloric acid (6 M, 50 μL). Samples were centrifuged for 5 min at 1800*g*, and tritiated substrate and product were separated by HPLC (HP1100, Agilent Technologies). The injection volume was 15 μL and the flow rate 0.8 mL/min on a Hichrom Ltd. C18 (5 μm, 4.6 mm × 150 mm) column using acetonitrile/H_2O (72:28) as the eluant. Detection was performed with a flow scintillation detector using an Ultima-Flo M (Packard) scintillator. Enzyme activity was quantified as area percentage of the product peak compared to the total area (substrate peak + product peak).

Mouse and Rat 11β-HSD1 SPA Assay. Enzyme assays were performed using purified recombinant mouse or rat 11β-HSD1. Assays were run in a total volume of 100 μL, including 40 μL of purified enzyme, 10 μL of compound A dilutions, 10 μL of [^{3}H]cortisone (200 nM final), 10 μL of NADPH (200 μM final), and 30 μL of assay buffer (50 mM Tris-HCl, 1 mM EDTA, pH 7.2). Assay was initiated by the addition of 40 μL of purified enzyme preparation (to achieve a final concentration of 20 nM). Assay plates were incubated on an orbital shaker for 1 h at rt. The reaction was stopped by the addition of 10 μL of buffer containing 100 μM of GA. At the same time, 10 μL of a 1:50 dilution of anticortisol (East Coast Biologics) and 100 μL of 15 mg/mL antimouse SPA beads were added to the wells.

Ex Vivo Mouse Pharmacodynamics. Male C57Bl/6 lean mice, 12 weeks old, were orally gavaged with vehicle (0.5% CMC/0.1% Tween 80 in H_2O) or **8b** at 30 mg/kg and sacrificed 2 and 6 h post dose, $n = 6$ per group. Inguinal fat pads and liver were removed and sectioned into three 30−40 mg samples and placed into 24-well Falcon plates containing prewarmed assay media that consisted of 1 μM cortisone in DMEM. Plates were then transferred to a 37 °C/CO_2 incubator and incubated for 2 h. Then cortisol product in the media was quantitated using a cortisol ELISA kit (Assay Designs Inc., Ann Arbor, MI). Enzyme activity is expressed as pg/mL of product formed per mg wet tissue weight. Data were analyzed for statistical significance (two-tailed *t* test) using GraphPad Prism 4.

X-ray Structure Determination. Human 11β-HSD1 (24−292) C272S mutant was expressed and purified as described previously.[39] The protein sample was concentrated to 5 mg/mL and passed through a Pierce Extracti-Gel D column. The inhibitor was dissolved in DMSO at 100 mM concentration and then added to the protein sample by 1:100 dilution. The enzyme was crystallized by vapor diffusion using hanging drops. The protein sample was mixed with an equal volume of well solution of 25% PEG 3350, 0.2 M ammonium acetate, and 0.1 M sodium citrate buffer (pH 5.6) at rt. The crystals belong to space group $P2_12_12_1$ with cell dimensions of $a = 49.03$, $b = 138.49$, and $c = 155.03$ Å, with four molecules in the asymmetric unit. Data were collected at beamline 5.0.2 at the Advanced Light Source using an ADSCQ315 detector and processed using HKL2000.[43] The phases were obtained by molecular replacement method using Molrep in CCP4 suite.[44] The model was built using the visualization program QUANTA (Accelrys). The model was refined using CNX[45] to a resolution of 3 Å with a crystallographic $R = 20.45\%$ and $R_{free} = 29.67\%$.

Supporting Information Available: Elemental analysis data, spectroscopic data, and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Zimmet, P. Z.; Alberti, K. G. M. M.; Shaw, J. Global and societal implications of the diabetes epidemic. *Nature* **2001**, *414*, 782–787.
(2) Horton, E. S. NIDDM: the devastating disease. *Diabetes Res. Clin. Pract.* **1995**, (Aug 28 Suppl.), S3–S11.
(3) Wajchenberg, B. L. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr. Rev.* **2000**, *21*, 697–738.
(4) Ross, S. A.; Gulve, E. A.; Wang, M. Chemistry and biochemistry of type 2 diabetes. *Chem. Rev.* **2004**, *104*, 1255–1282.
(5) Skyler, J. S. Diabetes mellitus: pathogenesis and treatment strategies. *J. Med. Chem.* **2004**, *47*, 4113–4117.
(6) Walker, B. R.; Seckl, J. R. 11β-Hydroxysteroid dehydrogenase type 1 as a novel therapeutic target in metabolic and neorodegenerative disease. *Expert Opin. Ther. Targets* **2003**, *7*, 771–783.
(7) Fotsch, C.; Askew, B. C.; Chen, J. J. 11β-Hydroxysteroid dehydrogenase-1 as a therapeutic target for metabolic diseases. *Expert Opin. Ther. Patents* **2005**, *15*, 289–303.
(8) Barf, T.; Williams, M. Recent progress in 11β-hydroxysteroid dehydrogenase type 1 (11-β- HSD1) inhibitor development. *Drugs Future* **2006**, *31*, 231–243.
(9) Krozowski, Z. 11β-Hydroxysteroid dehydrogenase and the short-chain alcohol dehydrogenase (SCAD) superfamily. *Mol. Cell. Endocrinol.* **1992**, *84*, C25−C31.
(10) Stewart, P. M.; Whorwood, C. B.; Walker, B. R. Steroid hormones and hypertension: the cortisol−cortisone shuttle. *Steroids* **1993**, *58*, 614–620.
(11) Rosmond, R. Role of stress in the pathogenesis of the metabolic syndrome. *Psychoneuroendocrinology* **2005**, *30*, 1–10.
(12) Asensio, C.; Muzzin, P.; Rohner-Jeanrenaud, F. Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance. *Int. J. Obes. Relat. Metab. Disord.* **2004**, *28* (Suppl. 4), S45–S52.
(13) Rask, E.; Olsson, T.; Söderberg, S.; Andrew, R.; Livingstone, D. E. W.; Johnson, O.; Walker, B. R. Tissue-specific dysregulation of cortisol metabolism in human obesity. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 1418–1421.
(14) Hanson, R. W.; Reshef, L. Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annu. Rev. Biochem.* **1997**, *66*, 581–611.
(15) Von Geldern, T. W.; Tu, N.; Kym, P. R.; Link, J. T.; Jae, H.-S.; Lai, C.; Apelqvist, T.; Rhonnstad, P.; Hagberg, L.; Koehler, K.; Grynfarb, M.; Goos-Nilsson, A.; Sandberg, J.; Österlund, M.; Barkhem, T.; Höglund, M.; Wang, J.; Fung, S.; Wilcox, D.; Nguyen, P.; Jakob, C.; Hutchins, C.; Färnegaårdh, M.; Kauppi, B.; Öhman, L.; Jacobson, P. B. Liver-selective glucocorticoid antagonists: a novel treatment for type 2 diabetes. *J. Med. Chem.* **2004**, *47*, 4213–4230.

(16) Bujalska, I. J.; Kumar, S.; Hewison, M.; Stewart, P. M. Differentiation of adipose stromal cells: the roles of glucocorticoids and 11β-hydroxysteroid dehydrogenase. *Endocrinology* **1999**, *140*, 3188–3196.

(17) Kotelevtsev, Y.; Holmes, M. C.; Burchell, A.; Houston, P. M.; Schmoll, D.; Jamieson, P.; Best, R.; Brown, R.; Edwards, C. R. W.; Seckl, J. R.; Mullins, J. J. 11β-Hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14924–14929.

(18) Masuzaki, H; Paterson, J.; Shinyama, H.; Morton, N. M.; Mullins, J. J.; Seckl, J. R.; Flier, J. S. A transgenic model of visceral obesity and the metabolic syndrome. *Science* **2001**, *294*, 2166–2170.

(19) Masuzaki, H.; Yamamoto, H.; Kenyon, C. J.; Elmquist, J. K.; Morton, N. M.; Paterson, J. M.; Shinyama, H.; Sharp, M. G. F.; Fleming, S.; Mullins, J. J.; Seckl, J. R.; Flier, J. S. Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. *J. Clin.Invest.* **2003**, *112*, 83–90.

(20) Alberts, P.; Engblom, L.; Edling, N.; Forsgren, M.; Klingström, G.; Larsson, C.; Rönquist-Nii, Y.; Öhman, B.; Abrahmsén, L. Selective inhibition of 11β-hydroxysteroid dehydrogenase type 1 decreases blood glucose concentrations in hyperglycaemic mice. *Diabetologia* **2002**, *45*, 1528–1532.

(21) Alberts, P.; Nilsson, C.; Selen, G.; Engblom, L. O. M.; Edling, N. H. M.; Norling, S.; Klingström, G.; Larsson, C.; Forsgren, M.; Ashkzari, M.; Nilsson, C. E.; Fiedler, M.; Bergqvist, E.; Öhman, B.; Björkstrand, E.; Abrahmsán, L. B. Selective inhibition of 11β-hydroxysteroid dehydrogenase type 1 improves hepatic insulin sensitivity in hyperglycemic mice strains. *Endocrinology* **2003**, *144*, 4755–4762.

(22) Ross, E. J.; Linch, D. C. Cushing's syndrome—killing disease: discriminatory value of signs and symptoms aiding early diagnosis. *Lancet* **1982**, *320*, 646–649.

(23) Wang, M. Inhibitors of 11β-hydroxysteroid dehydrogenase type 1 for the treatment of the metabolic syndrome. *Curr. Opin. Invest. Drugs* **2006**, *7*, 319–323.

(24) Chrousos, G. P. Is 11β-hydroxysteroid dehydrogenase type 1 a good therapeutic target for blockade of glucocorticoid actions? *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 6329–6330.

(25) Stewart, P. M.; Wallace, A. M.; Valentino, R.; Burt, D.; Shackleton, C. H. L.; Edwards, C. R. W. Mineralocorticoid activity of liquorice: 11β-hydroxysteroid dehydrogenase deficiency comes of age. *Lancet* **1987**, *330*, 821–824.

(26) (a) Barf, T.; Vallgårda, J.; Emond, R.; Häggström, C.; Kurz, G.; Nygren, A.; Larwood, V.; Mosialou, E.; Axelsson, K.; Olsson, R.; Engblom, L.; Edling, N.; Rönquist-Nii, Y.; öhman, B.; Alberts, P.; Abrahmsén, L. Arylsulfonamidothiazoles as a new class of potential antidiabetic drugs: discovery of potent and selective inhibitors of the 11β-hydroxysteroid dehydrogenase type 1. *J. Med. Chem.* **2002**, *45*, 3813–3815. (b) St. Jean, D. J., Jr.; Yuan, C.; Bercot, E. A.; Cupples, R.; Chen, M.; Fretland, J.; Hale, C.; Hungate, R. W.; Komorowski, R.; Veniant, M.; Wang, M.; Zhang, X.; Fotsch, C. 2-(*S*)-Phenethylaminothiazolones as potent, orally efficacious inhibitors of 11β-hydroxysteroid dehydrogenasetype 1. *J. Med. Chem.* **2007**, *50*, 429–432. (c) Flyrén, K.; Bergquist, L. O.; Castro, V. M.; Fotsch, C.; Johansson, L.; St. Jean, D.; Sutin, L.; Williams, M. Piperidine amides as 11β-hydroxysteroid dehydrogenase type 1 inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3421–3425. (d) Sutin, L.; Andersson, S.; Bergquist, L.; Castro, V. M.; Danielsson, E.; James, S.; Henriksson, M.; Johansson, L.; Kaiser, C.; Flyrén, K.; Williams, M. Oxazolones as potent inhibitors of 11β-hydroxysteroid dehydrogenase type 1. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4837–4840. (e) Yuan, C.; St. Jean, D. J., Jr.; Liu, Q.; Cai, L.; Li, A.; Han, B.; Moniz, G.; Askew, B.; Hungate, R. W.; Johansson, L.; Tedenborg, L.; Pyring, D.; Williams, M.; Wang, M.; Hale, C.; Chen, M.; Cupples, R.; Zhang, J.; Jordan, S.; Bartberger, M. D.; Sun, Y.; Emery, M.; Fotsch, C. The discovery of 2-anilinothiazolones as 11β-HSD1 inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *22*, 6056–6061.

(27) Xiang, J.; Ipek, M.; Suri, V.; Massefski, W.; Pan, N.; Ge, Y.; Tam, M.; Xing, Y.; Tobin, J. F.; Xu, X.; Tam, S. Synthesis and biological evaluation of sulfonamidooxazoles and β-keto sulfones: selective inhibitors of 11β-hydroxysteroid dehydrogenase type I. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2865–2869.

(28) (a) Olson, S.; Aster, S. D.; Brown, K.; Carbin, L.; Graham, D. W.; Hermanowski-Vosatka, A.; LeGrand, C. B.; Mundt, S. S.; Robbins, M. A.; Schaeffer, J. M.; Slossberg, L. H.; Szymonifka, M. J.; Thieringer, R.; Wright, S. D.; Balkovec, J. M. Adamantyl triazoles as selective inhibitors of 11β-hydroxysteroid dehydrogenase type 1. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4359–4362. (b) Gu, X.; Dragovic, J.; Koo, G. C.; Koprak, S. L.; LeGrand, C.; Mundt, S. S.; Shah, K.; Springer, M. S.; Tan, E. Y.; Thieringer, R.; Hermanowski-Vosatka, A.; Zokian, H. J.; Balkovec, J. M.; Waddell, S. T. Discovery of 4-heteroarylbicyclo[2.2.2]octyltriazoles as potent and selective inhibitors of 11β-HSD1: novel therapeutic agents for the treatment of metabolic syndrome. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5266–5269.

(29) Coppola, G. M.; Kukkola, P. J.; Stanton, J. L.; Neubert, A. D.; Marcopulos, N.; Bilci, N. A.; Wang, H.; Tomaselli, H. C.; Tan, J.; Aicher, T.; Knorr, D. C.; Jeng, A. Y.; Dardik, B.; Chatelain, R. E. Perhydroquinolylbenzamides as novel inhibitors of 11β-hydroxysteroid dehydrogenase type 1. *J. Med. Chem.* **2005**, *48*, 6696–6712.

(30) Su, X.; Vicker, N.; Ganeshapillai, D.; Smith, A.; Purohit, A.; Reed, M. J.; Potter, B. V. L. Benzothiazole derivatives as novel inhibitors of human 11β-hydroxysteroid dehydrogenase type 1. *Mol. Cell. Endocrinol.* **2006**, *248*, 214–217.

(31) (a) Yeh, V. S. C.; Kurukulasuriya, R.; Kerdesky, F. A. A highly efficient synthesis of potent and selective butyrolactam inhibitors of 11β-HSD1. *Org. Lett.* **2006**, *8*, 3963–3966. (b) Yeh, V. S. C.; Kurukulasuriya, R.; Madar, D.; Patel, J. R.; Fung, S.; Monzon, K.; Chiou, W.; Wang, J.; Jacobson, P.; Sham, H. L.; Link, J. T. Synthesis and structural activity relationship of 11β-HSD1 inhibitors with novel adamantane replacements. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5408–5413. (c) Yeh, V. S. C.; Patel, J. R.; Yong, H.; Kurukulasuriya, R.; Fung, S.; Monzon, K.; Chiou, W.; Wang, J.; Stolarik, D.; Imade, H.; Beno, D.; Brune, M.; Jacobson, P.; Sham, H.; Link, J. T. Synthesis and biological evaluation of heterocycle containing adamantane 11β-HSD1 inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5414–5419. (d) Yeh, V. S. C.; Yong, H.; Kurukulasuriya, R.; Fung, S.; Monzon, K.; Chiou, W.; Wang, J.; Stolarik, D.; Imade, H.; Shapiro, R.; Knourek-Segel, V.; Bush, E.; Wilcox, D.; Nguyen, P. T.; Brune, M.; Jacobson, P.; Link, J. T. Discovery of orally active butyrolactam 11β-HSD1 inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5555–5560. (e) Rohde, J. J.; Pliushchev, M. A.; Sorensen, B. K.; Wodka, D.; Shuai, Q.; Wang, J.; Fung, S.; Monzon, K. M.; Chiou, W. J.; Pan, L.; Deng, X.; Chovan, L. E.; Ramaiya, A.; Mullally, M.; Henry, R. F.; Stolarik, D. F.; Imade, H. M.; Marsh, K. C.; Beno, D. W. A.; Fey, T. A.; Droz, B. A.; Brune, M. E.; Camp, H. S.; Sham, H. L.; Frevert, E. U.; Jacobson, P. B.; Link, J. T. Discovery and metabolic stabilization of potent and selective 2-amino-β-(adamant-2-yl) acetamide 11β-hydroxysteroid dehydrogenase type 1 inhibitors. *J. Med. Chem.* **2007**, *50*, 149–164.

(32) Schuster, D.; Maurer, E. M.; Laggner, C.; Nashev, L. G.; Wilckens, T.; Langer, T.; Odermatt, A. The discovery of new 11β-hydroxysteroid dehydrogenase type 1 inhibitors by common feature pharmacophore modeling and virtual screening. *J. Med. Chem.* **2006**, *49*, 3454–3466.

(33) Webster, S. P.; Warda, P.; Binnie, M.; Craigie, E.; McConnell, K. M. M.; Sooy, K.; Vinter, A.; Seckl, J. R.; Walker, B. R. Discovery and biological evaluation of adamantyl amide 11β-HSD1 inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2838–2843.

(34) Andreash, R. Über einige Thioharnstoffderivate. *Chem. Ber.* **1898**, *31*, 137–139.

(35) (a) Hell, C. Über eine neue Bromierungsmethode organischer Säuren. *Ber. Dtsch. Chem. Ges.* **1881**, *14*, 891–893. (b) Volhard, J. Über die Darstellung α-Bromierter Säuren. *Justus Liebigs Ann. Chem.* **1887**, *242*, 141–163. (c) Zelinksy, N. Über eine bequeme Darstellungsweise von α-Bromopropionsäureestern. *Ber. Dtsch. Chem. Ges.* **1887**, *20*, 2026.

(36) Selvakumar, N.; Srinivas, D.; Khera, M. K.; Kumar, M. S.; Mamidi, R. N. V. S.; Sarnaik, H.; Charavaryamath, C.; Rao, B. S.; Raheem, M.; Das, J.; Iqbal, J.; Rajagopalan, R. Synthesis of conformationally constrained analogues of linezolid: structure—activity relationship (SAR) studies on selected novel tricyclic oxazolidinones. *J. Med. Chem.* **2002**, *45*, 3953–3962.

(37) Poss, M.; Iwanowicz, E.; Reid, J.; Lin, J.; Gu, Z. A mild and efficient method for the preparation of guanidines. *Tetrahedron Lett.* **1992**, *33*, 5933–5936.

(38) (a) Boehme, W. R. Stereochemistry of Diels—Alder adducts. III. Preparation and rearrangement of some brominated derivatives of norbornanecarboxylic acids. *J. Am. Chem. Soc.* **1959**, *81*, 2762–2765. (b) Kwart, H.; Null, G. The course of bromination in bicyclo[2.2.1] heptanecarboxylic acids; the observation of rearrangement during a reduction of β-bromoacids. *J. Am. Chem. Soc.* **1959**, *81*, 2765–2771. (c) Neuman, R.C., Jr.; Grow, R. H.; Binegar, G. A.; Gunderson, H. J. *cis*-Diazenes. *J. Org. Chem.* **1990**, *55*, 2682–2688.

(39) Zhang, J.; Osslund, T. D.; Plant, M. H.; Clogston, C. L.; Nybo, R. E.; Xiong, F.; Delaney, J. M.; Jordan, S. R. Crystal structure of murine 11β-hydroxysteroid dehydrogenase 1: an important therapeutic target for diabetes. *Biochemistry* **2005**, *44*, 6948–6957.

(40) Ogg, D.; Elleby, B.; Norstroem, C.; Stefansson, K.; Abrahmsen, L.; Oppermann, U.; Svensson, S. The crystal structure of guinea pig 11β-hydroxysteroid dehydrogenase type 1 provides a model for enzyme—lipid bilayer interactions. *J. Biol. Chem.* **2005**, *280*, 3789–3794.

(41) Hosfield, D. J.; Wu, Y; Skene, R. J.; Hilgers, M.; Jennings, A.; Snell, G. P.; Aertgeerts, K. Conformational flexibility in crystal structures

of human 11β-hydroxysteroid dehydrogenase type 1 provides insights into glucocorticoid interconversion and enzyme regulation. *J. Biol. Chem.* **2005**, *280*, 4639–4648.

(42) Elleby, B.; Svensson, S.; Wu, X.; Stefansson, K.; Nilsson, J.; Hallen, D.; Oppermann, U.; Abrahmsen, L. High-level production and optimization of monodispersity of 11β-hydroxysteroid dehydrogenase type 1. *Biochim. Biophys. Acta* **2004**, *1700*, 199–207.

(43) OtwinowskiZ.; Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **1997**, *276*, 307–326.

(44) Bailey, S. The ccp4 suite: programs for protein crystallography. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1994**, *50*, 760–763.

(45) Brunger, A. T.; Adams, P. D.; Clore, G. M.; Delano, W. L.; Gros, P.; Grossekunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. Crystallography and NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1998**, *54*, 905–921.

JM701551J