Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 4481–4487 **4481**
DOI: 10.1021/jm100242d

Discovery of a Potent, Orally Active 11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitor for Clinical Study: Identification of (*S*)-2-((1*S*,2*S*,4*R*)-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropyl-5-methylthiazol-4(5*H*)-one (AMG 221)

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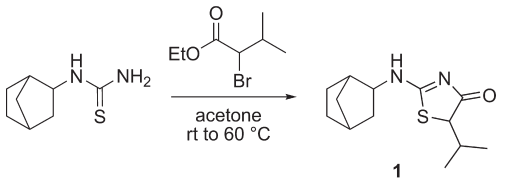
Received February 23, 2010

Thiazolones with an *exo*-norbornylamine at the 2-position and an isopropyl group on the 5-position are potent 11β-HSD1 inhibitors. However, the C-5 center was prone to epimerization in vitro and in vivo, forming a less potent diastereomer. A methyl group was added to the C-5 position to eliminate epimerization, leading to the discovery of (*S*)-2-((1*S*,2*S*,4*R*)-bicyclo[2.2.1]heptan-2-ylamino)-5-isopropyl-5-methylthiazol-4(5*H*)-one (AMG 221). This compound decreased fed blood glucose and insulin levels and reduced body weight in diet-induced obesity mice.

Introduction

Glucocorticoids have many physiological functions, such as augmentation of hepatic glucose production and suppression of glucose utilization in skeletal muscle and adipose tissue.[1] Glucocorticoid excess in Cushing's syndrome causes metabolic abnormalities similar to the features of the metabolic syndrome,[2] suggesting that glucocorticoid action may be involved in the development of these disorders. Thus, blockade of glucocorticoid action is considered a viable strategy to treat the metabolic syndrome.[3,4] 11β-Hydroxysteroid dehydrogenase (11β-HSD1[a]) is a bidirectional enzyme in vitro with both dehydrogenase and reductase activities.[5,6] However, in tissues and intact cells, it acts predominately as an oxoreductase, converting inert cortisone to active cortisol in humans or 11-dehydrocorticosterone (11-DHC) to corticosterone in rodents.[7−10] The isozyme of 11β-HSD1, 11β-HSD2, is a dehydrogenase and catalyzes the opposite reaction. 11β-HSD1 is primarily expressed in liver, adipose tissue, and brain, while 11β-HSD2 is mainly expressed in kidney. Since glucocorticoid activation of the mineralocorticoid receptor (MR) in kidney leads to hypertension, 11β-HSD2 activity protects MR from activation by inactivating cortisol. The adipose expression of 11β-HSD1 in obese human subjects is elevated,[11,12] suggesting that there is glucocorticoid excess in adipose tissues of these subjects, which could be linked to the development of obesity and insulin resistance. Transgenic overexpression of 11β-HSD1 in mouse adipose tissue resulted in the development of obesity, insulin resistance, dyslipidemia, and hypertension.[13,14] Moreover, mice with genetic ablation of 11β-HSD1 became resistant to diet-induced obesity (DIO), hyperglycemia, and dyslipidemia.[15,16] These data suggest that selective inhibition of 11β-HSD1 could have therapeutic value for the treatment of metabolic syndrome. In addition, liver-selective overexpression of 11β-HSD1 only led to mild insulin resistance and dyslipidemia but without obesity.[17] These findings also indicate that adipose is the primary target tissue for 11β-HSD1 inhibition.[1] A number of pharmaceutical and biotechnology companies have developed 11β-HSD1 inhibitors for the treatment of type 2 diabetes, including Biovitrum,[18] Abbott,[19,20] Merck,[21,22] Pfizer,[23−25] and Incyte.[26] Recently, clinical studies with an 11β-HSD1 inhibitor in patients with type 2 diabetes have provided additional evidence in support of the therapeutic value of this target.[26]

Scheme 1



Discussion

Through SAR work on our thiazolone series of compounds described in a previous report,[27] a lead compound, **1**, was identified. Compound **1**, prepared by the method outlined in Scheme 1, was a mixture of eight diastereomers with a

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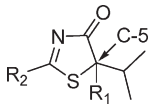
[a] Abbreviations: 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; 11-DHC, 11-dehydrocorticosterone; DIO, diet-induced obesity; GR, glucocorticoid receptor; GTT, glucose tolerance test; SGF, simulated gastric fluid ; SIF, simulated intestinal fluid.

Published on Web 05/13/2010

calculated K_i of 58 nM. We prepared all of the (±) *exo*-norbornyl and (±) *endo*-norbornyl analogues separately to identify the most potent diastereomer (see Supporting Information). The four (±) *endo*-norbornyl analogues had calculated K_i values in the range of 400−5000 nM and, therefore, were not further pursued. One of the *exo*-norbornyl diastereomers had better potency compared with the other three diastereomers (Table 1). The most potent *exo*-diastereomer was the (*S*,*S*) analogue, **2**, where the stereochemical assignment refers to the C-2 position of the norbornyl ring and the C-5 position of the thiazolone ring, respectively (see Table 1). We suspected that the C-5 position might be prone to epimerization. Incubation of compound **2** with either simulated gastric fluid (SGF) or simulated intestinal fluid (SIF) led to the formation of a mixture of compound **2** and its less active epimer, **3** (Figure 1). The formation of **3** in SGF is more pronounced than in SIF (Figure 1). This finding suggests that **2** may undergo epimerization within the gastrointestinal tract after oral administration.

Table 1. Human 11β-HSD1 Activities of Compounds[a]

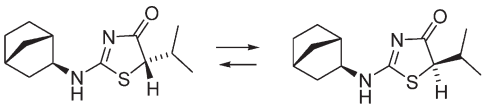


Compound	R_2 =	C-5 stereochem	R_1 =	SPA Ki (nM)[27]	Cell IC_{50} (nM)[27]
2		(*S*)	H	12.2 ± 0.6	15.3 ± 0.9
4		(*S*)	H	770 ± 59	1505 ± 152
3		(*R*)	H	329 ± 135	885 ± 84
5		(*R*)	H	227 ± 48	931 ± 584
7	 (±) exo	Mixture of (*R*) and (*S*) diastereomers	CH_3	17.3 ± 7.9	12.6 ± 16.0
8 (AMG 221)		(*S*)	CH_3	12.8 ± 1.7	10.1 ± 6.2
9		(*S*)	CH_3	27.8 ± 3.0	28.6 ± 20.1
10		(*R*)	CH_3	30.6 ± 3.7	56.8 ± 13.7
11		(*R*)	CH_3	47.2 ± 5.6	62.0 ± 35.7

[a] The absolute configuration of compounds **2**, **8**, and **9** were confirmed by X-ray analysis. Data are expressed as the mean ± SEM.

To evaluate if compound **2** undergoes epimerization in plasma, it was incubated with human or rat plasma and the sample mixture was then analyzed for the presence of **2** and its less active isomer, **3**. In fact, at the end of the incubation, the isomer was found in both human and rat plasma samples, indicating that the C-5 position on the thiazolone ring was susceptible to epimerization (Figure 2). In vitro epimerization of compound **2** readily occurred in both human and rat plasma (Figure 2). Incubation of compound **3** in rat and human plasma showed similar results with the formation of compound **2** (data not shown). The epimerization of **2** to **3** was also confirmed in vivo in the rat. Following intravenous administration of 2 mg/kg **2** to rats, small but measurable concentrations of **3** were observed (Figure 3). In contrast, there was a relatively greater amount of **3** formed following oral administration of 10 mg/kg compound **2** to rats (Figure 3). The mean AUC ratio of **3** to **2** following the iv dose was 8.5%, whereas following the oral dose a mean AUC ratio of 39% was observed. We hypothesized that oral administration might have resulted in greater epimerization of **2**, since it was exposed to acidic conditions in the stomach, which is consistent with the level of epimerization found in SGF (Figure 2).

To circumvent the epimerization problem, a methyl group was introduced at the C-5 position on analogue **6**, which produced a mixture of four diastereomers (**7**) (Scheme 2). This mixture had a K_i of 17.3 nM, and after separation of the four stereoisomers by chiral chromatography, the most potent compound was **8** (AMG 221) (K_i = 12.8 nM; see Table 1).[28] Compound **8** contained the (*S*,*S*) configuration at the C-2 position of the norbornyl and the C-5 position of the thiazolone, which was the configuration found in the most potent *des*-methyl analogue, compound **2**. Compound **8** was also potent in our cell-based assay and showed selectivity over



2 **3**

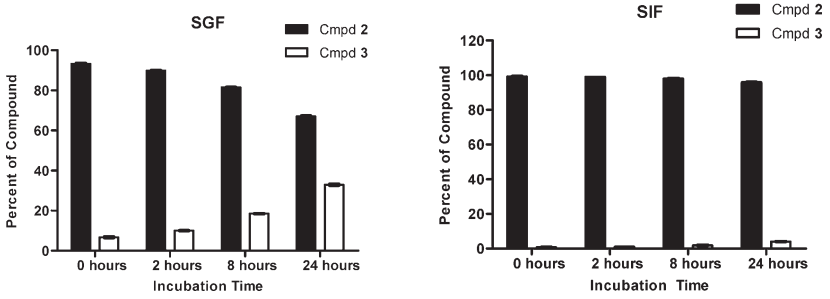


Figure 1. In vitro epimerization of **2** to **3** in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF).

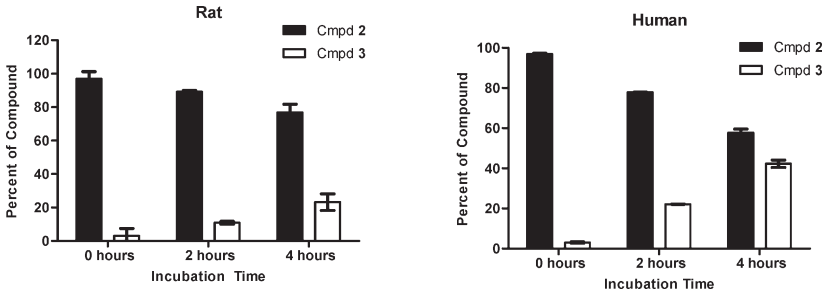


Figure 2. In vitro epimerization of **2** to **3** in rat and human plasmas.

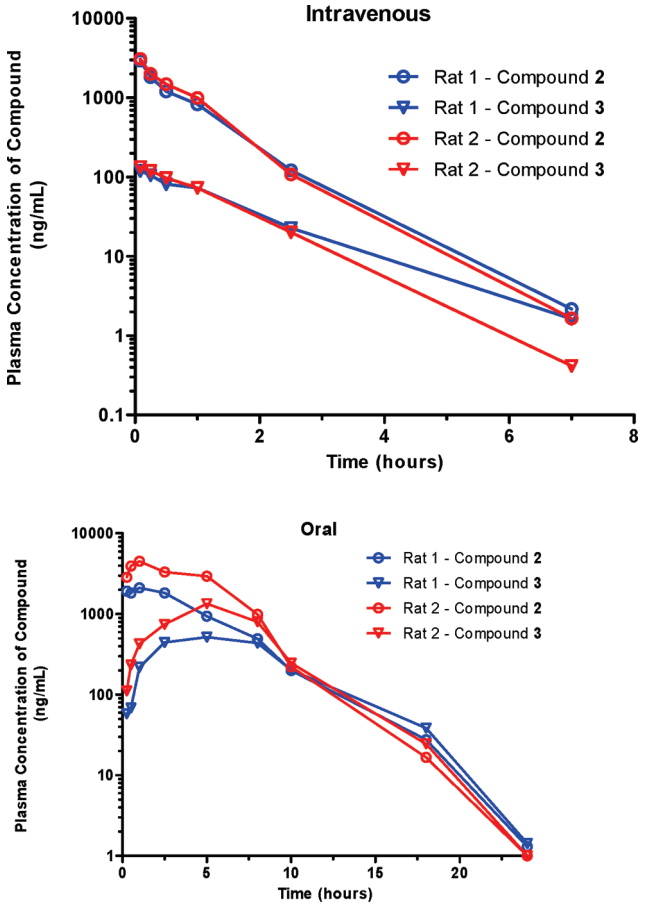


Figure 3. In vivo conversion of **2** to **3** in the rat following an intravenous (2 mg/kg) or oral dose (10 mg/kg) of **2**.

11β-HSD2, 17β-HSD1, and glucocorticoid receptor (GR) (IC_{50} values for all assays were >10 μM). This compound was also selective over 61 other human targets (less than 25% binding at 10 μM, data not shown). The cocrystal structure of **8** with the human enzyme was resolved. The overall fold of the enzyme was virtually the same as that previously published.[27] The binding mode of the compound and the arrangement of the active site residues were almost identical to an analogue complex structure reported previously[27] (PDB code 3BYZ) (Figure 4). The tautomerization state of the aminothiazolone core of **8**, possessing an endocyclic C=N double bond and exocyclic NH, was the same as in this previous cocrystal structure[27] as was its hydrogen bonding pattern with nearby residues. A hydrogen bonding interaction was observed between the thiazolone carbonyl oxygen atom and the backbone NH of Ala172. This was augmented by an additional hydrogen bond between the exocyclic NH of the inhibitor and the

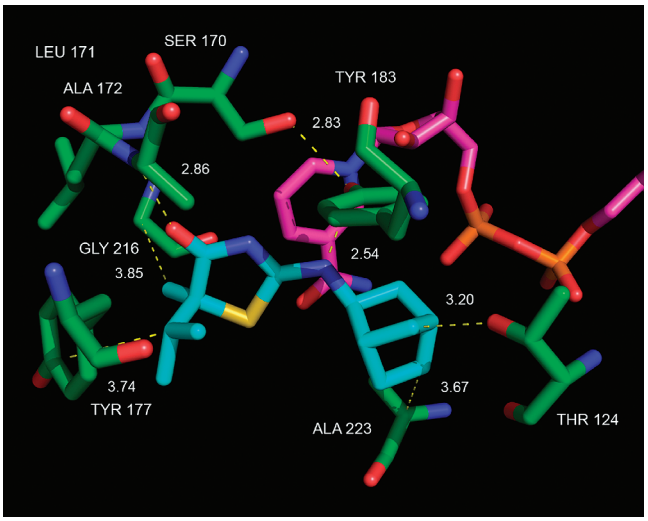
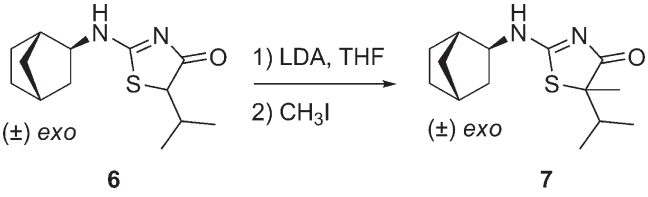


Figure 4. Key binding interactions of **8** with human 11β-HSD1. Carbon atoms of 11β-HSD1 are in green, NADPH in magenta, and **8** in cyan. Relevant hydrogen bonding and van der Waals interatomic distances are reported in ångströms.

Scheme 2



6 7

Table 2. Preclinical Pharmacokinetic Properties of Compound **8** in Animals[a]

	CD1 mouse	SD rat	beagle dog	cynomolgus monkey
CL ((L/h)/kg)	3.16	1.36	0.94	1.89
V_{ss} (L/kg)	1.00	1.73	1.02	0.98
$t_{1/2}$ (h)	15.6[b]	41.5[b]	8.1[b]	0.6
F (%)	54	34	50	7

[a] iv dose and formulation: mouse (5 mg/kg), rat (2 mg/kg), dog (3 mg/kg), and monkey (2 mg/kg) in 10% dimethylacetamide, 23% water, and 67% PEG 400. po dose and formulation: mouse (30 mg/kg), rat (10 mg/kg), and monkey (10 mg/kg) in 0.1% Tween-80 and 0.5% CMC in water; dog (10 mg/kg) in 10% dimethylacetamide, 23% water, and 67% PEG 400. [b] Terminal $t_{1/2}$.

side chain oxygen of Tyr183, the latter forming a second hydrogen bonding interaction with the Ser170 side chain. An alternative binding mode involving the exocyclic double bond tautomer (endocyclic NH) accompanied by a 180° reversal of the aminothiazolone core in the binding site has been observed[29] (PDB: 2RBE). The 2-norbonyl moiety of **8** occupied

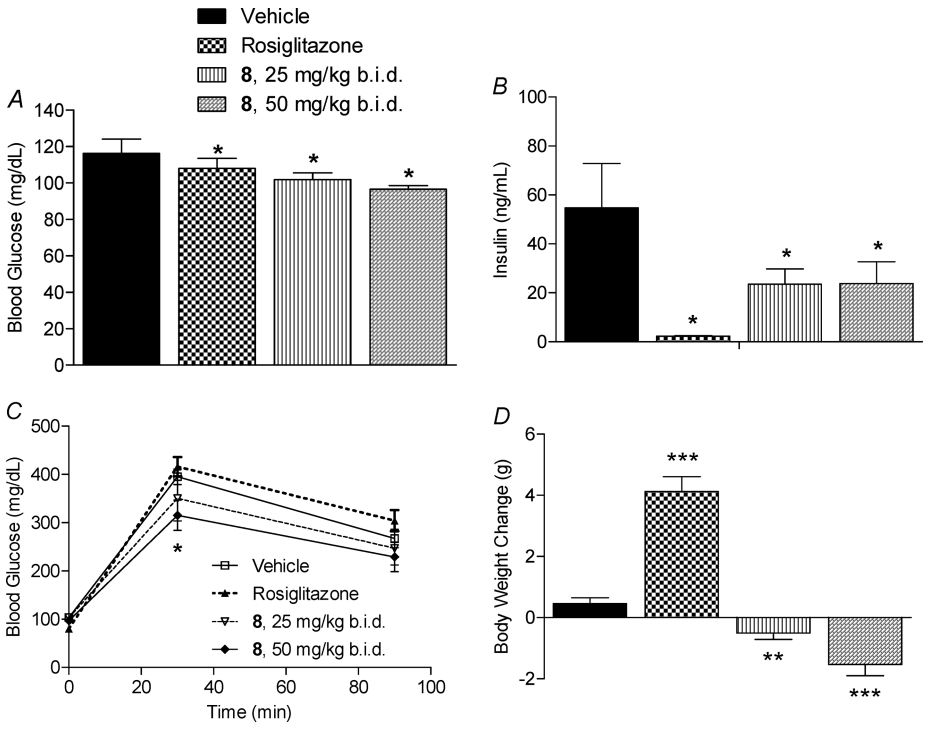


Figure 5. Effects of **8** on glucose and insulin levels, glucose tolerance test (GTT), and body weight in DIO mice: (A) blood glucose levels measured after 13 days of treatment (*, $P < 0.05$ vs vehicle); (B) plasma insulin levels measured after 13 days of treatment (*, $P < 0.05$ vs vehicle); (C) intraperitoneal GTT performed in DIO mice treated with vehicle, rosiglitazone (5 (mg/kg)/day, q.d.), or **8** at 25 or 50 mg/kg b.i.d.; (D) body weight measured at the end of the treatment period (**, $P \leq 0.01$ vs vehicle; ***, $P < 0.001$ vs vehicle).

a similar area of space as the cycloalkyl moiety in our previously published inhibitor complex 3BYZ.[27]

The PK profiles of **8** were evaluated in several animal species, including mouse, rat, dog, and monkey. The in vivo pharmacokinetic properties are summarized in Table 2. The data from a 5 mg/kg iv study in mice are reported for ease of comparison with those in other species, since the same vehicle was used (Table 2). Mouse iv PK was also carried out at 2 mg/kg, and similar CL and V_{ss} values were obtained (data not shown). The plasma clearance of **8** was moderate to high in mouse, rat, monkey, and dog relative to hepatic blood flow (Table 2). Disposition was biphasic where the compound exhibited a rapid initial phase of decline which accounted for the majority of the area under the concentration−time profile followed by the appearance of a "deep" compartment characterized by a much longer terminal half-life in all the species tested. Compound **8** had a good bioavailability in mouse, rat, and dog. However, the bioavailability in monkey was low, which could be attributed to first-pass hepatic metabolism consistent with low liver microsomal stability. This compound did not inhibit any of the five main cytochrome P450 enzymes, 3A4, 2D6, 1A2, 2C9, and 2C19 ($IC_{50} > 15$ μM). The in vitro K_m for metabolism was greater than 100 μM and there was no evidence for transport by P-glycoprotein.

Compound **8** was administered in a mouse pharmacodynamic model measuring 11β-HSD1 activity in an adipose tissue explant as described previously.[27] 11β-HSD1 activity was inhibited by 33%, 55%, and 47% in the inguinal fat at 4 h after **8** was orally gavaged at 5, 15, and 50 mg/kg, respectively. At 8 h, the 11β-HSD1 activity in the inguinal fat of the 5 mg/kg group had returned to a level (~10% inhibition) close to that in the control animals treated with vehicle, but there was still significant inhibition in the 15 and 50 mg/kg groups (36% and 39% inhibition, respectively). To test the pharmacological effects of **8** in a disease animal model, we administered this compound at 25 and 50 mg/kg, b.i.d., in DIO mice for 13−14 days. Administering **8** b.i.d. was necessary to inhibit 11β-HSD1 activity in the fat for at least 8 h per dose so that the total inhibition period within a day was more than 16 h. At the end of the study, fed blood glucose showed statistically significant reduction in comparison to the vehicle group (Figure 5A). On day 13, there were statistically significant decreases in insulin levels in all treated groups when compared with the vehicle control group (Figure 5B). On day 14 and after a 12 h fast, glucose tolerance was slightly improved in the compound **8** treatment groups compared with the vehicle group (Figure 5C). As expected, mice treated for only 13 days with rosiglitazone did not show improved glucose tolerance. Also, as shown from the baseline of the glucose tolerance test, fasted blood glucose levels of compound **8** treated mice were not changed compared to the vehicle treated mice. Body weight was also decreased in a dose-dependent fashion in mice treated with **8** compared to the control group (Figure 5D). Also, as expected, body weight of mice treated with rosiglitazone was statistically significantly increased.

Conclusion

We identified a potent, selective 11β-HSD1 inhibitor compound **2**. However, since this compound underwent both in vitro and in vivo epimerization, it was not suitable for further advancement. Through structural modification of this compound to prevent epimerization, **8** was discovered. This compound had acceptable potency, selectivity, PK properties, and pharmacological efficacy. The favorable profile of compound **8** justified preclinical safety studies, after which it was advanced to clinical trials.

Experimental section

Preparation of Compounds. (*R*,*S*)-2-((±)-*exo*-Bicyclo[2.2.1]-heptan-2-ylamino)-5-isopropylthiazol-4(5*H*)-one. A mixture of

(±)-*exo*-1-(bicyclo[2.2.1]heptan-2-yl)thiourea (530 mg, 3.1 mmol), (*S*)-2-bromo-3-methylbutanoic acid (560 mg, 3.1 mmol), and sodium acetate (310 mg, 3.7 mmol) in anhydrous ethanol (10 mL) was heated in a round-bottomed flask to reflux under nitrogen. After 4.5 h, the reaction mixture was cooled to room temperature, and the mixture was concentrated in vacuo. To the residue was added ethyl acetate (20 mL), which was washed with water (20 mL) and brine (20 mL). The organic layer was then dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (4:1 hexanes/acetone) to yield the desired product as a colorless oil (355 mg, 45%). The mixture was further purified by SFC (supercritical fluid chromatography method, column OD-H (4.6 mm × 15 cm, 5 μm), flow rate of 2.8 mL/min, 89% CO_2 (l) and 11% ethanol, T = 35 °C, back pressure of 120 bar) to give the four diastereomers described below.

(*S*)-2-((1*S*,2*S*,4*R*)-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropylthiazol-4(5*H*)-one (2). Yield = 41 mg, 5.2%. ^{1}H NMR ($CDCl_3$ + 1 drop of TFA, 400 MHz): δ 4.35 (d, J = 4.02 Hz, 1 H), 3.42 (m, 1 H), 2.68 (m, 1 H), 2.48 (br s, 2 H), 1.90 (ddd, J = 2.26, 8.03, 13.3 Hz, 1 H), 1.69 (m, 2 H), 1.585 (br d, J = 12 Hz, 2 H), 1.38 (br d, J = 10.5 Hz, 1 H), 1.23 (m, 2 H), 1.16 (d, J = 7.03 Hz, 3 H), 1.05 (d, J = 6.52 Hz, 3 H). MS (ESI) m/z: 253.2 (M + 1). Anal. Calcd for $C_{13}H_{20}N_2OS$: C, 61.87; H, 7.99; N, 11.10. Found: C, 62.01; H, 7.96; N, 11.08.

(*R*)-2-((1*S*,2*S*,4*R*)-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropylthiazol-4(5*H*)-one (3). Yield = 24 mg, 3.1%. ^{1}H NMR ($CDCl_3$ + 1 drop of TFA, 400 MHz): δ 4.36 (d, J = 3.51 Hz, 1 H), 3.44 (br d, J = 5.52 Hz, 1 H), 2.67 (m, 1 H), 2.48 (m, 2 H), 1.92 (ddd, J = 2.01, 8.03, 13.6 Hz, 1 H), 1.71−1.54 (m, 4 H), 1.375 (br d, J = 12 Hz, 1 H), 1.22 (m, 2 H), 1.16 (d, J = 6.52 Hz, 3 H), 1.04 (d, J = 6.52 Hz, 3 H). MS (ESI) m/z: 253.1 (M + 1). Anal. Calcd for $C_{13}H_{20}N_2OS$: C, 61.87; H, 7.99; N, 11.10. Found: C, 61.94; H, 7.88; N, 11.09.

(*R*)-2-((1*R*,2*R*,4*S*)-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropylthiazol-4(5*H*)-one (4). Yield = 34 mg, 4.3%. ^{1}H NMR ($CDCl_3$ + 1 drop of TFA, 400 MHz): δ 4.33 (t, J = 3.76 Hz, 1 H), 3.42 (d, J = 7.53, 1 H), 2.68 (m, 1 H), 2.48 (br s, 2 H), 1.90 (dd, J = 8.03, 13.1 Hz, 1 H), 1.73−1.58 (m, 4 H), 1.38 (d, J = 10.5 Hz, 1 H), 1.22 (m, 2 H), 1.16 (d, J = 6.52 Hz, 3 H), 1.05 (d, J = 6.52 Hz, 3 H). MS (ESI) m/z: 253.2 (M + 1). HPLC (method A (5 min)) R_f = 1.81 min, 100, 100% (215, 254 nm); (method B (15 min)) R_f = 8.72 min, 100, 100% (215, 254 nm).

(*S*)-2-((1*R*,2*R*,4*S*)-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropylthiazol-4(5*H*)-one (5). Yield = 24 mg, 3.1%. ^{1}H NMR ($CDCl_3$ + 1 drop of TFA, 400 MHz): δ 4.34 (d, J = 3.51 Hz, 1 H), 3.43 (d, J = 5.02, 1 H), 2.66 (m, 1 H), 2.47 (br s, 2 H), 1.91 (ddd, J = 2.26, 7.91, 13.4 Hz, 1 H), 1.71−1.56 (m, 4 H), 1.37 (d, J = 10.6 Hz, 1 H), 1.24 (m, 2 H), 1.15 (d, J = 7.03 Hz, 3 H), 1.04 (d, J = 6.52 Hz, 3 H). MS (ESI) m/z: 253.2 (M + 1). HPLC (method A (5 min)) R_f = 1.82 min, 100, 100% (215, 254 nm); (method B (15 min)) R_f = 8.73 min, 100, 100% (215, 254 nm).

(*R*,*S*)-2-((±)-*exo*-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropyl-5-methylthiazol-4(5*H*)-one. To a 250 mL round-bottomed flask equipped with magnetic stirring was added diisopropylamine (8.0 mL, 57 mmol) in 100 mL of anhydrous THF. After the reaction mixture was cooled to −78 °C under an inert atmosphere, *n*-butyllithium (34 mL of a 1.6 M solution in hexanes, 54 mmol) was added dropwise over 15 min. Following the complete addition of *n*-butyllithium, the reaction mixture was stirred for an additional 40 min, and then (*R*,*S*)-2-((±)-*exo*-bicyclo[2.2.1]heptan-2-ylamino)-5-isopropylthiazol-4(5*H*)-one (2.2 g, 8.7 mmol) in 20 mL of anhydrous THF was added over 5 min. After ~1.5 h, methyl iodide (2.2 mL, 35 mmol) was added, and the reaction mixture was stirred for another 5.5 h at −78 °C. Brine (60 mL) was then added, and the reaction flask was allowed to warm to room temperature. The organic layer was then separated from the aqueous layer, and the aqueous layer was extracted with THF (2×). The organic layers were combined and dried over $MgSO_4$, filtered, and concentrated in vacuo. Following flash chromatography (hexanes/ethyl acetate) the desired product was isolated as a white solid (2.37 g). The mixture was further purified by SFC (supercritical fluid chromatography method, two columns run in series: AD-H (4.6 mm ×15 cm, 5 μm) and OD-H (4.6 mm × 15 cm, 5 μm) (flow rate of 3 mL/min, 90% CO_2 (l) and 10% ethanol, T = 40 °C, back pressure of 120 bar).

(*S*)-2-((1*S*,2*S*,4*R*)-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropyl-5-methylthiazol-4(5*H*)-one (8). Yield = 210 mg, 9%. ^{1}H NMR ($CDCl_3$ + 1 drop of TFA, 400 MHz): δ 3.40 (br d, J = 5 Hz, 1 H), 2.46 (m, 2 H), 2.34 (dq, J = 6.61, 6.78 Hz, 1 H), 1.89 (ddd, J = 2.01, 8.03, 13.5 Hz, 1 H), 1.78 (s, 3 H), 1.69 (m, 2 H), 1.58 (m, 2 H), 1.38 (br d, J = 11.0 Hz, 1 H), 1.22 (m, 2 H), 1.12 (d, J = 7.03 Hz, 3 H), 1.025 (d, J = 6.52 Hz, 3 H). MS (ESI) m/z: 267.2 (M + 1). Anal. Calcd for $C_{14}H_{22}N_2OS$: C, 63.12; H, 8.32; N, 10.52. Found: C, 63.31; H, 8.29; N, 10.59.

(*S*)-2-((1*R*,2*R*,4*S*)-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropyl-5-methylthiazol-4(5*H*)-one (9). Yield = 240 mg, 10%. ^{1}H NMR ($CDCl_3$ + 1 drop of TFA, 400 MHz): δ 3.39 (dd, J = 2.26, 7.78 Hz, 1 H), 2.47 (m, 2 H), 2.33 (sept, J = 6.69 Hz, 1 H), 1.90 (ddd, J = 2.26, 8.03, 13.3 Hz, 1 H), 1.78 (s, 3 H), 1.71−1.55 (m, 4 H), 1.37 (br d, J = 10.0 Hz, 1 H), 1.22 (m, 2 H), 1.10 (d, J = 6.52 Hz, 3 H), 1.00 (d, J = 6.52 Hz, 3 H). MS (ESI) m/z: 267.2 (M + 1). Anal. Calcd for $C_{14}H_{22}N_2OS$: C, 63.12; H, 8.32; N, 10.52. Found: C, 63.34; H, 8.39; N, 10.54.

(*R*)-2-((1*S*,2*S*,4*R*)-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropyl-5-methylthiazol-4(5*H*)-one (10). Yield = 260 mg, 11%. ^{1}H NMR ($CDCl_3$ + 1 drop of TFA, 400 MHz): δ 3.41 (dd, J = 2.26, 8.28 Hz, 1 H), 2.47 (m, 2 H), 2.33 (dq, J = 6.61, 6.78 Hz, 1 H), 1.91 (ddd, J = 2.01, 7.53, 13.6 Hz, 1 H), 1.79 (s, 3 H), 1.69−1.56 (m, 4 H), 1.37 (br d, J = 10.5 Hz, 1 H), 1.21 (m, 2 H), 1.11 (d, J = 7.03 Hz, 3 H), 1.01 (d, J = 6.52 Hz, 3 H). MS (ESI) m/z: 267.2 (M + 1). HPLC (method A (5 min)) R_f = 1.94 min, 100, 96% (215, 254 nm); (method B (15 min)) R_f = 10.3 min, 99, 96% (215, 254 nm).

(*R*)-2-((1*R*,2*R*,4*S*)-Bicyclo[2.2.1]heptan-2-ylamino)-5-isopropyl-5-methylthiazol-4(5*H*)-one (11). Yield = 233 mg, 10%. ^{1}H NMR ($CDCl_3$ + 1 drop of TFA, 400 MHz): δ 3.39 (dd, J = 3.40, 5.02 Hz, 1 H), 2.46 (m, 2 H), 2.34 (dq, J = 6.61, 6.78 Hz, 1 H), 1.89 (ddd, J = 2.26, 7.91, 13.4 Hz, 1 H), 1.77 (s, 3 H), 1.72−1.55 (m, 4 H), 1.38 (br d, J = 10.5 Hz, 1 H), 1.21 (m, 2 H), 1.11 (d, J = 6.52 Hz, 3 H), 1.02 (d, J = 6.52 Hz, 3 H). MS (ESI) m/z: 267.2 (M + 1). Anal. Calcd for $C_{14}H_{22}N_2OS$: C, 63.12; H, 8.32; N, 10.52. Found: C, 63.06; H, 8.27; N, 10.58.

Purity of all final compounds were >95% as determined by LC−MS and/or elemental analysis.

Epimerization of Compounds in Vitro and in Vivo. Epimerization study was conducted in both simulated gastric fluid (SGF, which contains 2 g of NaCl, 3.2 g of pepsin, and 7 mL of HCl QS to 1000 mL of water, pH 1.12) and simulated intestinal fluid (SIF, which contains 6.8 g of potassium phosphate monobasic, 190 mL of 0.2 N NaOH, 10 g of pancreatin QS to 1000 mL of water, pH 7.5 at 23 °C). The study was conducted by preparing a 3 mL solution of compound **2** at 0.1 mg/mL in SIF and SGF. The solution was maintained in a water bath at 37 °C. Samples of 0.25 mL were withdrawn at each time point, and the reaction was quenched by adding acetonitrile. Samples were centrifuged, and the supernatant was analyzed by LC−MS.

Compounds **3** and **2** were incubated in rat and human plasma at 37 °C for 24 h. Aliquots of plasma were taken at 0, 6, and 24 h for measurement of the corresponding **2** or its diastereomer **3** for assessment of epimerization at the C-5 position on the thiazolone ring. To determine the extent of epimerization in vivo, Sprague−Dawley rats (n = 2/group) were administered either an intravenous (2 mg/kg) or oral (10 mg/kg) dose of **2**. Blood was collected for the measurement of **3** and **2** predose and at intervals up to 24 h to characterize the pharmacokinetics. Compounds **3** and **2** were analyzed in plasma using HPLC to separate the diastereomers and then detected using electrospray ionization (positive ion) MS/MS (API 4000, Applied Biosystems) and single ion monitoring (SIM). The assay quantitation limit was 2 ng/mL for **2** and 1 ng/mL for **3**.

Crystal Structure. The X-ray structure of the complex of **8** and human 11β-HSD1 was determined to a resolution of 2.6 Å using a published protocol.[27] The final R and R_{free} are 21.7% and 30.0%, respectively.

PK Studies. Compound **8** was administered via po or iv to mouse, rat, or monkey. Blood samples were collected at various time points, and compound concentrations were measured.

Ex Vivo Assay. The assay was carried out as described previously.[27]

Animals for Pharmacology Studies. Three-week-old male C57BL/6 mice were fed high fat (HF) diet chow (D12492 high fat diet, Research Diets Inc., New Brunswick, NJ) for 12 weeks (age of mice at start of experiment was 15−16 weeks) to induce insulin resistance and obesity. Animals were maintained at a constant temperature and had free access to food and drinking water in a light-dark cycle with lights on from 6:30 a.m. to 6:30 p.m.

Pharmacology Study of 8 in Diet-Induced Obesity (DIO) Mice. Mice were sorted into groups of n = 11−12 to provide equal distribution of pretreatment glucose levels and body weight between groups. Fed blood glucose and insulin levels were measured before starting the treatment and at the end of the treatment. Mice were orally gavaged with **8** at doses of 50 or 100 (mg/kg)/day twice a day (i.e., 25 or 50 mg/kg twice a day with half dose in the morning and half in the afternoon) or with vehicle (0.1% Tween-80 and 0.5% CMC in water) for 13 or 14 days. Rosiglitazone was included in the study and delivered at ∼5 mg/kg/day in HF diet chow for 13 or 14 days.

After 13 days of treatment, each treatment group was split into two post-treatment subgroups, subgroup A with n = 5 and subgroup B with n = 6−7. From subgroup A, plasma was collected for measurements of fed blood glucose and insulin levels (blood was also collected from all animals at baseline [day − 1] for the same measurements). For subgroups B, 14 days after starting treatments, glucose tolerance testing (GTT, 2 g/kg of body weight intraperitoneal glucose injection) was performed after a 12 h fast.

Intraperitoneal GTT was carried out in DIO mice fasted for 12 h (9 p.m. to 9 a.m.). Fasted blood glucose was measured prior to an ip injection of glucose (0.5 g/mL dextrose) at 2 g/kg body weight. Blood samples were collected from the retro-orbital sinuses of nonanesthetized mice. In order to minimize stress levels in the tested animals, only 30 and 90 min blood glucose levels were measured using the OneTouch profile meter.

Blood Chemistry Measurements. Blood glucose levels were measured immediately following blood collection on a glucose monitor (OneTouch profile meter, Lifescan, Inc., Milpitas, CA). Plasma insulin was measured by radioimmunoassay using a kit from Linco Research (St. Charles, MO).

11β-HSD1 Enzymatic Assays. In vitro biochemical scintillation proximity assay (SPA) and cell-based assays are as described previously.[27]

Statistical Analyses. All statistical analyses were carried out using a two-tailed Student's t test assuming unequal variances (GraphPad Prism software; San Diego, CA).

Supporting Information Available: Preparation of (*S*)-2-bromo-3-methylbutanoic acid and (±)-*exo*-1-(bicyclo[2.2.1]heptan-2-yl)thiourea, details of the HPLC conditions used, details of the stereochemical determination, and X-ray data. This material is available free of charge via the Internet at http://pubs.acs.org.

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