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Spirocyclic ureas: Orally bioavailable 11β-HSD1 inhibitors identified by computer-aided drug design

Colin M. Tice *, Wei Zhao, Zhenrong Xu, Salvacion T. Cacatian, Robert D. Simpson, Yuan-Jie Ye, Suresh B. Singh, Brian M. McKeever, Peter Lindblom, Joan Guo, Paula M. Krosky, Barbara A. Kruk, Jennifer Berbaum, Richard K. Harrison, Judith J. Johnson, Yuri Bukhtiyarov, Reshma Panemangalore, Boyd B. Scott, Yi Zhao, Joseph G. Bruno, Linghang Zhuang, Gerard M. McGeehan, Wei He, David A. Claremon

Vitae Pharmaceuticals, 502 West Office Center Drive, Fort Washington, PA 19034, USA

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ABSTRACT

Structure-guided drug design led to the identification of a class of spirocyclic ureas which potently inhibit human 11β-HSD1 in vitro. Lead compound **10j** was shown to be orally bioavailable in three species, distributed into adipose tissue in the mouse, and its (R) isomer **10j2** was efficacious in a primate pharmacodynamic model.

11β-hydroxysteroid dehydrogenase (11β-HSD1) is a member of the short chain dehydrogenase/reductase (SDR) superfamily. 11β-HSD1 increases local tissue concentrations of the active glucocorticoid cortisol by NADPH-dependent reduction of inactive cortisone. It is primarily expressed in liver and adipose tissue, and its elevated expression in adipose tissue has been linked to obesity, insulin resistance, diabetes and cardiovascular disease[1–5] in humans. Moreover, the phenotype of transgenic mice overexpressing 11β-HSD1 in adipose tissue includes visceral obesity, insulin resistance and hypertension,[6,7] while 11β-HSD1 knockout mice are resistant to diet induced obesity and have increased insulin sensitivity.[8–10] Thus, a selective inhibitor of 11β-HSD1 may be useful for the treatment of diabetes and has the potential for positive effects on multiple cardiovascular risk parameters.

Carbenoxolone (**1**, Fig. 1), a semisynthetic derivative of the natural product 18β-glycyrrhetinic acid, is a nonselective inhibitor of 11β-HSD1 and of 11β-HSD2, which catalyzes the NAD-dependent oxidation of cortisol to cortisone. Despite its lack of selectivity for 11β-HSD1, **1** has been investigated in animal and human studies where it improved insulin sensitivity.[11,12] Since 2002,[13] more selective, synthetic inhibitors of 11β-HSD1 have been reported.[14–16] Triazole **2** increased insulin sensitivity and decreased fasting glucose, cholesterol, and adipose tissue mass in mice.[17] Oral dosing of thiazolone **3** has been reported to increase plasma adiponectin levels and decrease fasting glucose levels in KKA$^{\gamma}$ mice.[18,19]

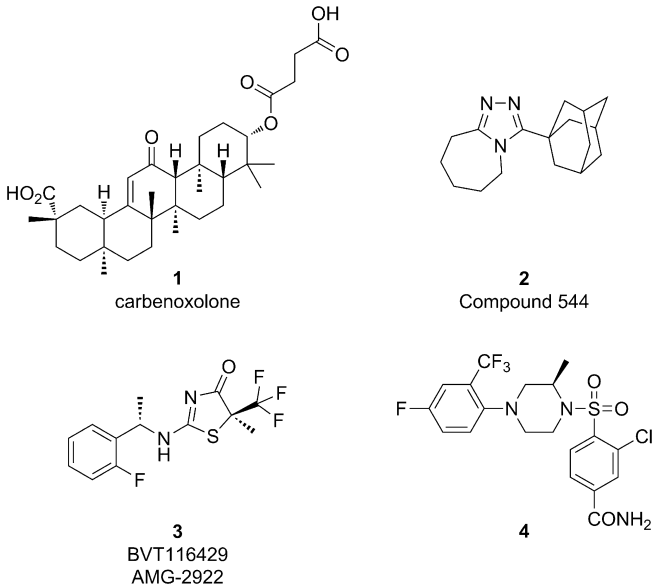

Figure 1. Literature 11β-HSD1 inhibitors.

* Corresponding author. Tel.: +1 215 461 2042; fax: +1 215 461 2006.
E-mail address: ctice@vitaerx.com (C.M. Tice).

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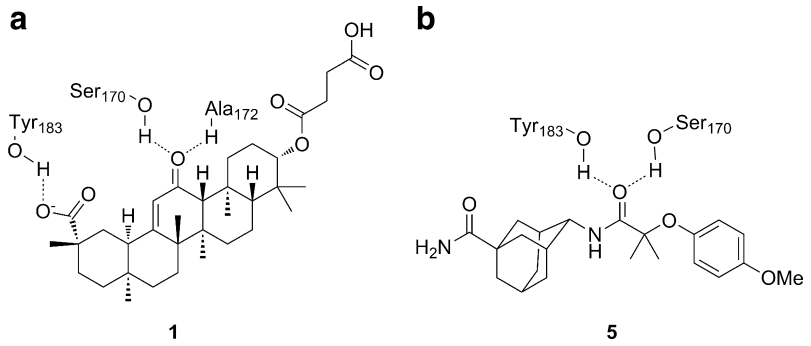


Figure 2. Protein ligand hydrogen bonding interactions. (a) Carbenoxolone **1** based on 2bel, (b) *N*-(2-adamantyl)amide **5** based on 2irw.

Animal studies have been reported on a number of sulfonamide inhibitors.[20–22] For example, piperazine sulfonamide **4** reduced fed glucose and fasted insulin in mice when incoporated into a high fat diet at 30 mg/kg/day.[22] The activity of these compounds in animal models further validates the potential for selective inhibitors of 11β-HSD1 in the treatment of diabetes.

Prior to initiation of our medicinal chemistry program, we examined the publicly available crystal structures of 11β-HSD1, focusing on 2bel in which carbenoxolone (**1**) is bound to the human form of the enzyme.[23] The triad of residues Ser170, Tyr183 and Lys187 are characteristic of the SDR superfamily and are involved in the catalytic cycle of the enzyme. The side chain hydroxyl of Tyr183 donates a hydrogen bond to the E-ring carboxylate of ligand **1**, while the side chain hydroxyl of Ser170 and the backbone NH of Ala172 donate hydrogen bonds to the ketone oxygen of **1** (Fig. 2a). Further inspection revealed that the enzyme has large, primarily hydrophobic pockets on both sides of the catalytic triad. One of these (Pocket I) is formed by the NAD cofactor, Thr124, Leu126 and Val180. The second hydrophobic pocket (Pocket II) is formed by Leu126, Val180 and Tyr177. Because the B chain of the protein is truncated in 2bel, Pocket II appears to have a large opening to solvent; however, the physiologically active form of 11β-HSD1 is dimeric[24] and subsequent protein structures with longer B chain constructs have revealed a smaller opening to solvent formed by Asp217, Tyr280 and Tyr177.[25–30] In fact, residues from the C-terminus of the 'B' monomer form part of the ligand binding pocket of the 'A' monomer and vice versa.

Consistent with the presence of two spacious hydrophobic pockets, a number of adamantane containing 11β-HSD1 inhibitors have been reported in the literature.[31–34] Using Contour™, a proprietary structure-based drug design program, models of a number of known ligands incorporating an N-(2-adamantyl)amide moiety were generated within the binding site of 11β-HSD1. The most satisfactory poses were obtained with the adamantane ring system positioned in hydrophobic Pocket I and the carbonyl oxygen positioned between Ser170 and Tyr183 such that it accepted hydrogen bonds from the side chain hydroxyl groups of both amino acids (Fig. 2b). The amide NH, however, did not participate in a hydrogen bonding interaction. Subsequent to these designs, an X-ray structure of N-(2-adamantyl)amide **5** (Fig. 3) bound to human 11β-HSD1 (PDB code: 2irw) was published and confirmed the validity of our model.[25]

The model also predicted that ureas of 2-aminoadamantane would also be well accommodated within the binding site. Thus simple urea **6a** was prepared and had an enzyme IC_{50} of 16.1 nM (Table 1). Based on these observations, we initiated a medicinal chemistry program to improve potency, selectivity and physical properties of urea **6a**, enabled by additional modeling.

Ureas of general structures **6** and **10** were prepared from 2-aminoadamantane and commercially available piperidines **7** and spiropiperidine esters **8** (Scheme 1).[35] 2-Aminoadamantane was activated for urea formation as its isocyanate, its *p*-nitrophenylcarbamate or its (1-imidazolyl) carbonyl derivative. Hydrolysis of esters **9a–j** with LiOH gave carboxylic acid analogs **10a–j**. The esters of **9b** and **9j** were separated by chromatography on a chiral column and hydrolyzed to give the single enantiomers of **10b** and **10j**. Acylsulfonamide **10k** and carboxamide **10l** were prepared by EDC catalyzed reaction of acid **10j** with methanesulfonamide and ammonia, respectively. Alcohol **10m** was prepared by $LiAlH_4$ reduction of **10j**. The configuration of **10j2**, the more potent enantiomer of **10j**, was determined to be (R) based on X-ray crystallography of sulfonamide derivative **11** (Scheme 2).

Compounds were assayed for inhibition of 11β-HSD1 in enzyme- and cell-based assays. Both measured the conversion of [^{3}H]-cortisone to [^{3}H]-cortisol, which was quantified using SPA

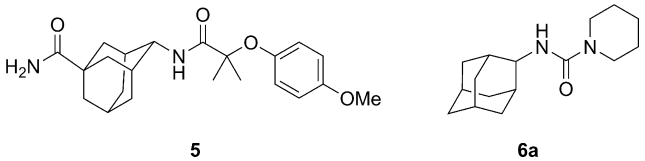
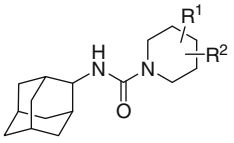
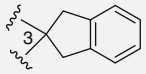
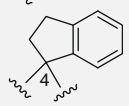
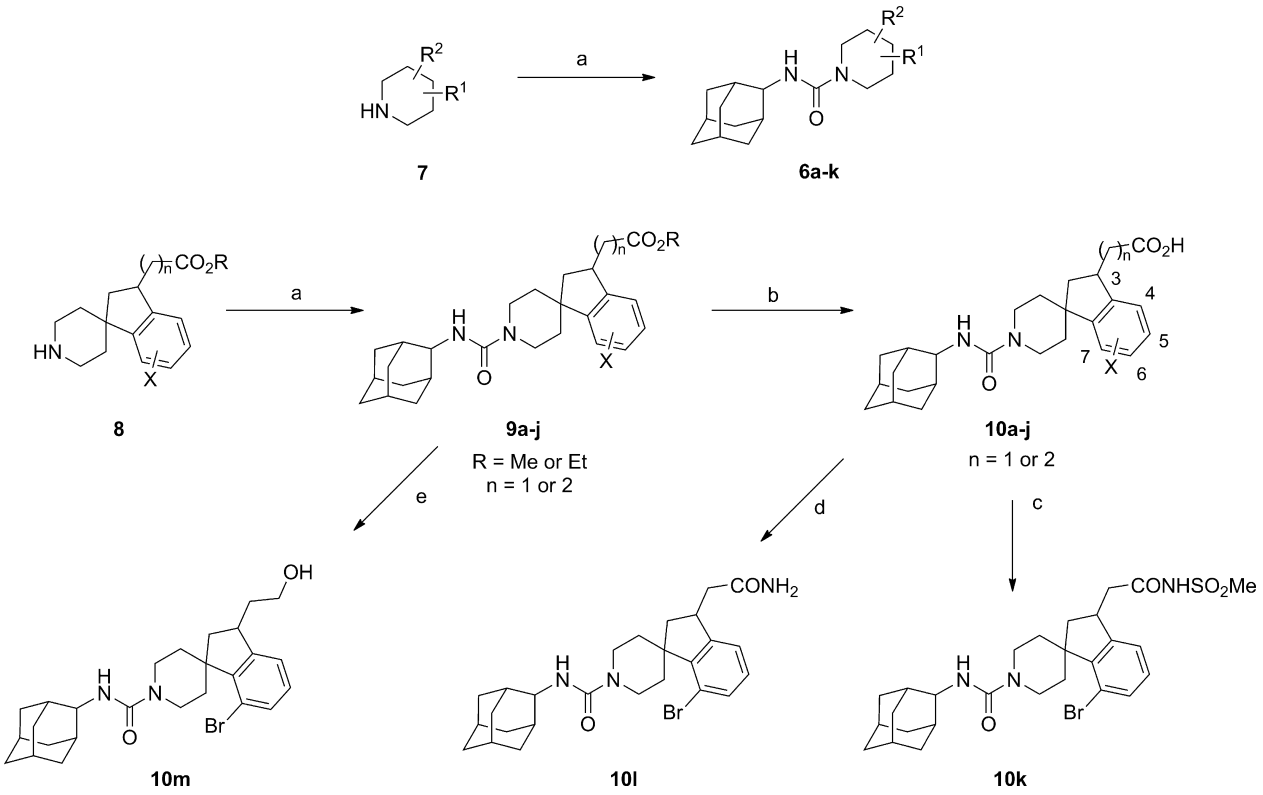


Figure 3. Adamantyl amide and urea inhibitors.

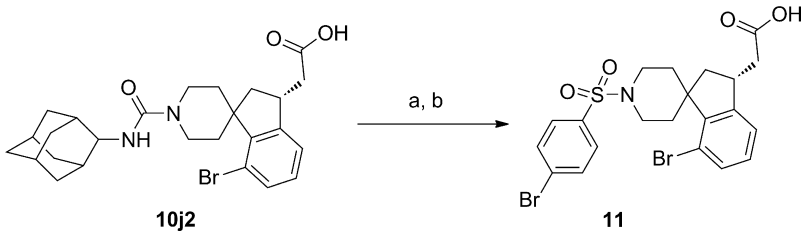
Table 1
SAR of adamantyl piperidine ureas **6**



Compd No.	R^1	R^2	Enzyme IC_{50} (nM)
6a	H	H	16.1
6b	2-Bn	H	1.4
6c	3-Bn	H	0.9
6d	4-Bn	H	1.1
6e	2-Ph	H	2.2
6f	3-Ph	H	0.8
6g	4-Ph	H	0.9
6h			0.7
6i			0.8
6j	4-Ph	4-$CONH_2$	41.6
6k	4-Ph	4-OH	56.8



Scheme 1. Synthesis of inhibitors **6** and **10**. R^1, R^2, X and *n* are as defined in Tables 1 and 2. Reagents and conditions: (a) 2-adamantyl isocyanate, *i*-Pr_2NEt, CH_2Cl_2 or *p*-nitrophenyl (2-adamantyl)carbamate, *i*-Pr_2NEt, CH_2Cl_2 or 2-aminoadamantane, carbonyl diimidazole, CH_2Cl_2; (b) LiOH, H_2O, MeOH; (c) $MeSO_2NH_2$, EDC, DMAP, CH_2Cl_2; (d) NH_3, EDC, HOBt, *i*-Pr_2NEt, CH_2Cl_2; (e) $LiAlH_4$, THF, 0 °C.



Scheme 2. Reagents and conditions: (a) *t*-BuONa, *n*-BuOH, μwave, 150 °C, 1 h; (b) 4-$BrC_6H_4SO_2Cl$, Et_3N, CH_2Cl_2, rt.

beads and a microscintillation plate reader.[36] Biochemical assays used recombinant 11β-HSD1 isolated as a microsomal preparation from transfected CHO cells. Assays were performed in 25 mM HEPES, pH 7.4, 50 mM KCl, 2.5 mM NaCl, 1 mM $MgCl_2$, 1 mM NADPH and 80 nM [^{3}H]-cortisone at room temperature for 1 h. Cell-based potency was assessed in differentiated human adipocytes by the addition of cortisone (80 nM [^{3}H]-cortisone) to the cell culture medium and incubation at 37 °C for 2 h. IC_{50} values represent the mean of at least duplicate assays and were generated from an 8-point dose-response curve.

Benzyl (**6b–6d**) and phenyl (**6e–6g**) groups substituted on all three positions of the piperidine ring of **6a** were able to fill Pocket II and improved enzyme potency to <2.5 nM (Table 1). Contour™ generated models indicated that 3-benzylpiperidine **6c** could be constrained into spirocycle **6h** and 4-phenylpiperidine urea **6g** could be constrained into spirocycle **6i**, both of which retained potency while providing rigid frameworks on which to append substitution.[37] While the enzyme potencies of **6b–6i** were attractive, their lipophilicity was higher than desirable for drug-like molecules. For example, compound **6i** has c log *D* = 4.35 and polar surface area of only 32 Å^2. The main goal for medicinal chemistry became lowering c log *D* and improving water solubility by introduction of polar groups into the molecule. To avoid introducing a

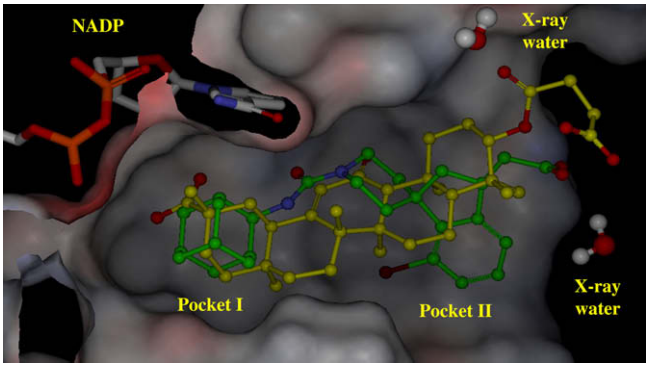
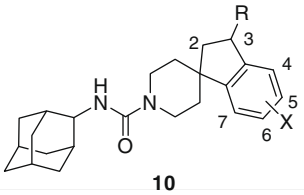


Figure 4. Overlap of carbenoxolone **1** and urea **10j2** in the binding site of 11β-HSD1. The protein structure, pose of **1** and crystallographic waters are taken from 2bel. The carbon atoms of **1** are depicted in gold. A model of **10j2**, generated by Contour™, is depicted in green. A change in the conformation of Tyr177 was necessary to accommodate **10j2**.

Table 2
SAR of spirocyclic ureas **10**



10

	R	X	Enzyme IC_{50} (nM)	Adipocyte IC_{50} (nM)	11β-HSD2 (nM)
6i	H	H	0.8	2.7	[e]
10a	CO_2H	H	68.6	>200	[e]
10b	CH_2CO_2H	H	16.1	60.3	>10,000
10b1[a]	CH_2CO_2H	H	39.9	193.5	5,900
10b2[b]	CH_2CO_2H	H	16.5	57.8	21,200
10c	CH_2CO_2H	4-Me	3.4	19.7	510
10d	CH_2CO_2H	5-Me	6.7	49.1	>10,000
10e	CH_2CO_2H	6-Me	8.9	22.5	>10,000
10f	CH_2CO_2H	7- Me	7.6	18.3	>10,000
10g	CH_2CO_2H	5-Cl	3.4	23.2	>10,000
10h	CH_2CO_2H	6-Cl	3.4	30.2	>10,000
10i	CH_2CO_2H	7-Cl	2.2	10.7	>10,000
10j	CH_2CO_2H	7-Br	1.6	5.3	>10,000
10j1[c]	CH_2CO_2H	7-Br	2.0	7.5	>10,000
10j2[d]	CH_2CO_2H	7-Br	1.1	2.5	>10,000
10k	$CH_2CONHSO_2Me$	7-Br	2.0	22.3	>10,000
10l	CH_2CONH_2	7-Br	0.5	0.9	[e]
10m	CH_2CH_2OH	7-Br	1.7	18.0	9,000

[a] Stereochemical configuration unknown: derived from enantiomer of methyl ester **9b** with shorter retention time on a Chiral Technologies ODH column eluted with a 90:10 hexane/*i*-PrOH mixture containing 0.22% Et_2NH.
[b] Stereochemical configuration unknown: derived from enantiomer of methyl ester **9b** with longer retention time on a Chiral Technologies ODH column eluted with a 90:10 hexane/*i*-PrOH mixture containing 0.22% Et_2NH.
[c] S isomer.
[d] R isomer.
[e] Not tested.

chiral center, we elected to make further modifications on the 4-substituted piperidine derivatives **6g** and **6i**.

Initial attempts to introduce polar functionality at the 4-position of **6g** led to less potent analogs **6j** and **6k**. This was consistent with our model in which the OH and $CONH_2$ groups of **6j** and **6k** lie within a hydrophobic region of the binding site. Comparison of the model of spirocycle **6i** bound to 11β-HSD1 with the X-ray structure of carbenoxolone (**1**) indicated that a carboxylic acid could be appended to C3 of the ethylene bridge of the spirocycle and reach the same water channel region as the succinate carboxylate of carbenoxolone (Fig. 4). In addition to imparting water solubility, a carboxylate functionality was anticipated to reduce target promiscuity.[38] On the other hand, carboxylic acids are frequently highly protein bound.[39]

Direct attachment of a carboxylic acid at C3 reduced enzyme potency by almost 100× (**10a** vs **6i**, Table 2); however, when an acetic acid moiety was appended at this position a less drastic 20× reduction in potency was observed (**10b** vs **10i**). Rat PK parameters of **10b** are shown in Table 3. The compound was rapidly absorbed and had high oral bioavailability but was subject to rapid clearance. Chiral chromatography of the methyl ester precursor **9b** permitted characterization of the individual enantiomers of **10b**. Although isomer **10b2**, derived from the methyl ester with the longer retention time, was slightly more potent in the adipocyte assay than its enantiomer **10b1**, the similar activity of the two enantiomers is consistent with our model in which the acetic acid side chain is accommodated in the solvent channel.

Examination of the models of both enantiomers of **10b** bound to the protein suggested that small lipophilic substituents would be tolerated at the 4-, 5-, 6- and 7-positions on the fused benzene ring which occupies Pocket II. The 7-position looked especially favorable since a suitable hydrophobic substituent at this position should pack against the hydrophobic residues Leu126 and Val180 and occupy a similar position to the methyl group in the 8-position of carbenoxolone. Appending methyl groups at the 4-, 5-, 6- and 7-

Table 3
Pharmacokinetic data for **10b** and **10j**[a,b,c]

Compound	Species	$AUC_{(inf)}$ (ng h/mL)	$t_{1/2}$ (h)	IV CL (mL/min kg)	*F* (%)
10b	Rat[c]	3532	2.87	47.1	94
10j	Rat[c]	5397	3.46	26.6	90
10j	Mouse[c]	1718	6.17	28.1	29
10j	Monkey[d]	743	6.03	13.8	31

[a] Oral PK parameters are shown unless otherwise stated.
[b] Compounds were dosed as sodium salts.
[c] Dose: 9.68 mg/kg PO; 1.9 mg/kg IV.
[d] Dose: 2 mg/kg PO, 1 mg/kg IV.

Table 4
Mouse tissue distribution of **10j** and **10j2**[a]

Compound	Drug level in liver (ng/mL)	Drug level in adipose (ng/mL)	Drug level in plasma (ng/mL)
10j	5130	77	442
10j2	7067	127	393

[a] Animals ($n = 3$) were dosed PO with 30 mg/kg suspended in 0.5% methocellulose in PBS. Tissues were harvested at 4 h and snap frozen. Later, thawed tissues were homogenized in 0.05 M BES, pH 7, and compound was extracted into acetonitrile. Concentrations were determined by LC–MS/MS, by comparison to a standard curve generated independently in each tissue matrix.

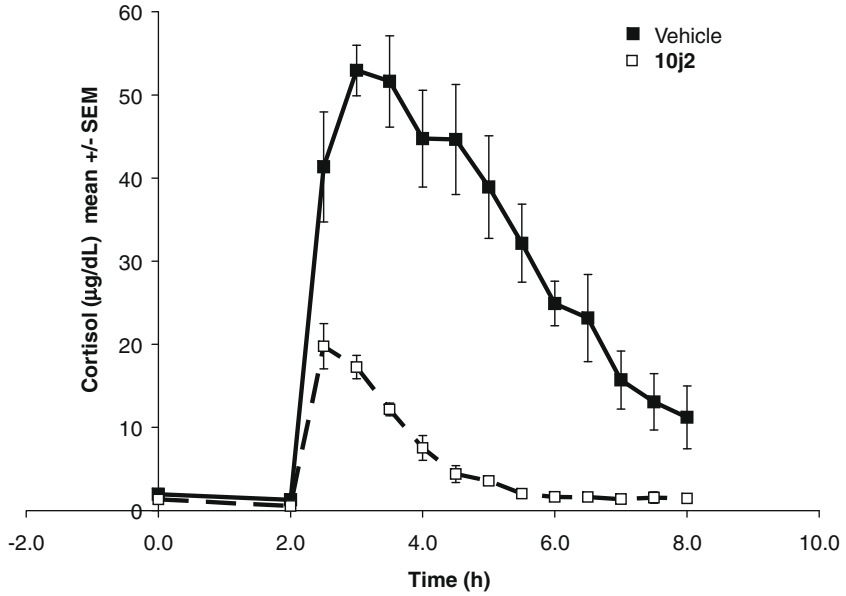


Figure 5. Pharmacodynamic effects of **10j2** in cynomolgus monkeys. In this model, the animals were dosed with dexamethasone 8 h prior to compound administration ($t = -8$ h) to suppress endogenous plasma cortisol concentrations. The animals, then, received vehicle or compound (10 mg/kg, PO) at time zero ($t = 0$ h) and two hours later ($t = 2$ h), were challenged with cortisone 21-acetate (25 mg, PO). Blood samples were drawn every 30 min for 6 h to measure cortisol levels. The animals were crossed over after a seven day washout.

positions (**10c**–**10f**) improved enzyme potency in all cases; however, methyl substitution at the 4-position led to an increase in 11β-HSD2 activity. Chlorine substitution had a favorable effect on potency at the 5-, 6- and, especially, 7-positions (**10g**–**10i**). Installation of a bromine at the 7-position gave **10j** with an IC_{50} of 1.6 nM in the enzyme assay and 5.3 nM in the cellular assay, a 2× improvement over the 7-chloro compound **10i**. Subsequently, the enantiomers of **10j** were separated and assayed individually: the R isomer, **10j2**, proved to be 3× more potent than the S isomer, **10j1**, in adipocytes. Three derivatives of the carboxylic acid moiety in **10j** were prepared. Introduction of the weakly acidic acylsulfonamide group in **10k** decreased cellular potency. In the neutral acetamide derivative **10l**, increased enzyme and cellular potency were demonstrated, while hydroxyethyl compound **10m** was less potent than **10j** in cells.

Bromo compound **10j** was selected for advanced in vitro evaluation. The c log *D* of **10j** is 3.1 and its PSA is 70 Å^2. It was >1000× selective for 11β-HSD1 over three other steroid dehydrogenases: 3β -HSD2, 11β-HSD2 and 17β-HSD2. Its IC_{50} values against recombinant CYP3A4, CYP2C9 and CYP2D6 were >15 μM, and its half-life in human liver microsomes was >1 h. The single enantiomer **10j2** did not significantly inhibit or bind to a panel of 68 receptors and ion channels in the MDS Pharma Services LeadProfilingScreen®.

Compound **10j** was profiled in pharmacokinetic (PK) and in pharmacodynamic (PD) studies in vivo: **10j** had high bioavailability (%*F* = 90) and moderate clearance (26.6 mL/min·kg) in the rat (Table 3). Oral bioavailability was more modest in mouse (%*F* = 29) and monkey (%*F* = 31). Tissue distribution studies in mice showed that after oral administration of **10j** and **10j2** (30 mg/kg), compound was detected in adipose and liver, the key target tissues (Table 4).[40] Compound **10j2** was tested in a cynomolgus monkey PD model using a crossover design ($n = 5$ animals). When dosed PO at 10 mg/kg, **10j2** reduced the plasma concentration of cortisol ~80% (Fig. 5), consistent with significant inhibition of 11β-HSD1, in vivo.

We have described the structure guided discovery of **10j2**, a potent and selective inhibitor of 11β-HSD1, in vitro. This compound is orally bioavailable in three species, distributes into adipose tissue in the mouse, and significantly inhibits 11β-HSD1 in a primate pharmacodynamic model.

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