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Synthesis and optimization of novel 4,4-disubstituted cyclohexylbenzamide derivatives as potent 11β-HSD1 inhibitors

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ABSTRACT

The synthesis and SAR of a series of 4,4-disubstituted cyclohexylbenzamide inhibitors of 11β-HSD1 are described. Optimization rapidly led to potent, highly selective, and orally bioavailable inhibitors demonstrating efficacy in both rat and non-human primate ex vivo pharmacodynamic models.

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a key enzyme that acts as an NADPH-dependent reductase capable of converting inactive glucocorticoids such as cortisone into their active form (e.g., cortisol) in specific tissues, such as liver, adipose, and brain.[1–4] Conversely, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), a structurally related isoenzyme of 11β-HSD1, catalyzes the conversion of cortisol to cortisone using $NADP^+$ as a cofactor. 11β-HSD2 is expressed in cells that contain the mineralocorticoid receptor (MR) and protects the MR by converting cortisol to cortisone.[5] Selective inhibition of 11β-HSD1 may be a viable therapeutic strategy for the treatment of metabolic syndrome and has attracted significant attention from the pharmaceutical research community.[6–29]

We previously described the design and optimization of substituted cyclohexylbenzamide 11β-HSD1 inhibitors.[30–32] During the course of that research, we noted that 4,4-disubstituted cyclohexylbenzamides, such as compound **1** (Fig. 1), exhibited significantly less potential for PXR transactivation relative to the corresponding 4-monosubstituted analogs. In addition, **1** showed excellent cross-species pharmacokinetics and in vivo inhibition of 11β-HSD1 in a rat ex vivo pharmacodynamic model. However, the potency of **1** made it unsuitable for further development in its present form. We felt that additional modifications of **1** at the 4-position of the cyclohexane ring would offer an opportunity to increase its potency while maintaining other properties (such as pharmacokinetics) which would provide a compound more suitable for development.

Compounds were synthesized via the routes outlined in Schemes 1–5.[33] 4,4-Disubstituted cyclohexyl benzamides **2**, **9**, **14**, **15**, and **16**, were prepared in a straightforward manner (Scheme 1). 4-Aryl/heteroaryl, 4-cyanocyclohexanones **19** were obtained by a

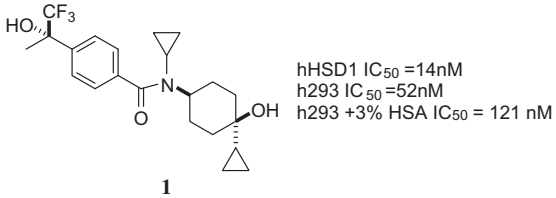

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Figure 1. An early 4,4-disubstituted cyclohexylbenzamide 11β-HSD1 inhibitor (**1**).

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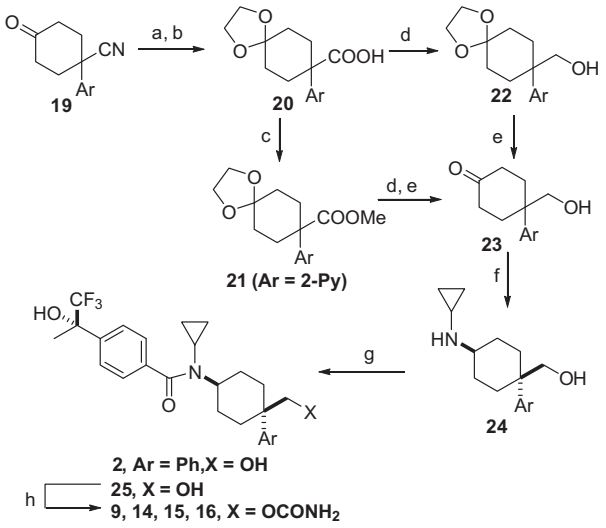
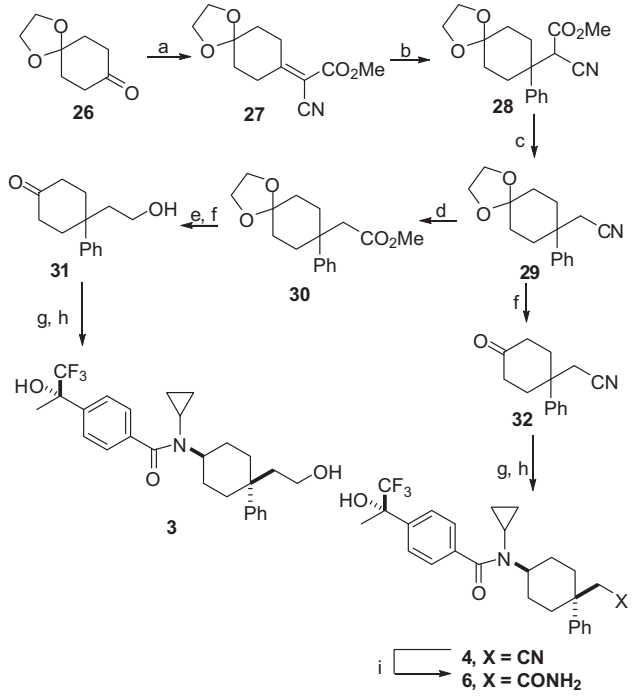
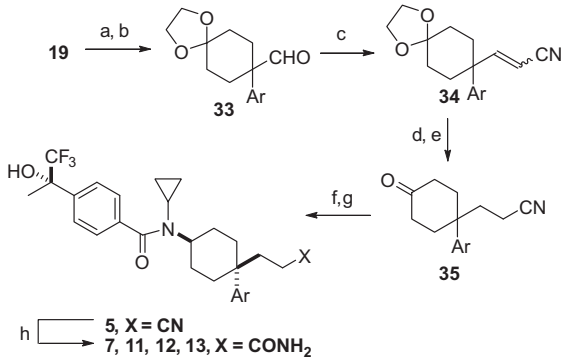


Scheme 1. Reagents and conditions: (a) ethylene glycol, *p*-toluenesulfonic acid, benzene, reflux, 45–92%; (b) ethylene glycol, KOH, 170 °C, 54–88%; (c) K_2CO_3, MeI, THF, 52–74%; (d) $LiAlH_4$, THF, reflux, 54–92%; (e) 3 N HCl, THF, 60–95%; (f) cyclopropylamine, $NaBH(OAc)_3$, acetic acid, dichloroethane, 8–24%; (g) EDC, HOAt, (*S*)-4-(1,1,1-trifluoro-2-hydroxypropan-2-yl)benzoic acid, DMF, 48–57%; (h) trichloroacetyl isocyanate, neutral Al_2O_3, $CHCl_3$, 84–95%.

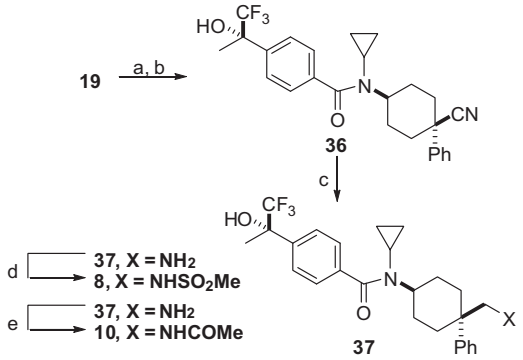


Scheme 2. Reagents and conditions: (a) ethyl cyanoacetate, acetic acid, NH_4OAc, toluene, reflux, 70%; (b) 2-bromobenzene, *n*-BuLi, CuI, *n*-Bu_2S, Et_2O, 49%; (c) DMSO, NaCl, H_2O, 80%; (d) (i) ethylene glycol, KOH, 170 °C, 54–92%; (ii) K_2CO_3, MeI, THF, 52–74%; (e) $LiAlH_4$, THF, 65–84%; (f) 3 N HCl, THF, 60–75%; (g) cyclopropylamine, $NaBH(OAc)_3$, acetic acid, dichloroethane, 21–35%; (h) EDC, HOAt, (*S*)-4-(1,1,1-trifluoro-2-hydroxypropan-2-yl)benzoic acid, DMF, 58–62%; (i) KOH, *t*-BuOH, 90 °C, 81%.

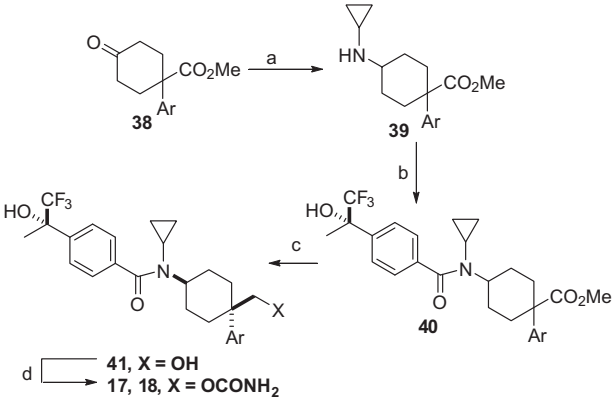
tandem one-pot double Michael addition–Dieckman condensation between methyl acrylate and the appropriate benzylic nitrile, followed by Krapcho decarboxylation.[34] Protection of **19** as its monoethylene ketal was followed by hydrolysis of the nitrile to produce acid **20**. Reduction of **20** with $LiAlH_4$ followed by removal of the ketal protecting group gave disubstituted cyclohexanones **23**.



Scheme 3. Reagents and conditions: (a) ethylene glycol, *p*-toluenesulfonic acid, benzene, reflux, 45–92%; (b) $LiAlH_4$ (0.5 equiv), THF, 80–98%; (c) NaH, diethyl (cyanomethyl) phosphonate, THF, 63–80%; (d) H_2, 10% Pd/C, EtOAc, 89–99%; (e) 3 N HCl, THF, 63–76%; (f) cyclopropylamine, $NaBH(OAc)_3$, acetic acid, dichloroethane, 33%; (g) EDC, HOAt, (*S*)-4-(1,1,1-trifluoro-2-hydroxypropan-2-yl)benzoic acid, DMF, 52%; (h) KOH, *t*-BuOH, 90 °C, 77%.



Scheme 4. Reagents and conditions: (a) cyclopropylamine, $NaBH(OAc)_3$, acetic acid, dichloroethane, 75–78%; (b) EDC, HOAt, (*S*)-4-(1,1,1-trifluoro-2-hydroxypropan-2-yl)benzoic acid, DMF, 49–57%; (c) H_2, Raney Ni, 28% NH_4OH, MeOH, 98%; (d) MsCl, Et_3N, 75%; (e) Hunig's base, acetyl chloride, DMF, 79%.



Scheme 5. Reagents and conditions: (a) cyclopropylamine, $NaBH(OAc)_3$, acetic acid, dichloroethane, 94%; (b) EDC, HOAt, (*S*)-4-(1,1,1-trifluoro-2-hydroxypropan-2-yl)benzoic acid, DMF, 74%; (c) $LiAlH_4$, THF, 21–31%; (d) trichloroacetyl isocyanate, neutral Al_2O_3, $CHCl_3$, 54%.

In a similar fashion, 2-pyridyl cyclohexanone **23** was prepared by conversion of **20** into ester **21** followed by reduction and deprotection. Reductive amination of **23** with cyclopropylamine resulted in a mixture of *cis*/*trans* cyclopropylamine products, and the desired

trans isomer **24** was separated by silica gel chromatography and coupled with (*S*)-4-(1,1,1-trifluoro-2-hydroxypropan-2-yl)benzoic acid[30] to afford primary alcohol **25** (**2**, Ar = Ph, X = OH), which was transformed into the corresponding carbamates **9**, **14**, **15**, and **16** using trichloroacetyl isocyanate.

Compounds **3** and **4** were synthesized via reductive amination of cyclohexanones **31** and **32** with cyclopropylamine followed by an amide coupling reaction (Scheme 2). Preparation of **31** and **32** proceeded through the monoethylene ketal of cyclohexadione **26**. Reaction of ketal **26** with ethyl cyanoacetate, followed by conjugate addition afforded intermediate **28**. Krapcho decarboxylation of **28** was followed by deprotection of the ketal gave cyclohexanone **32**. Intermediate **31** was prepared from **29** using a series of common transformations. Finally, hydrolysis of the cyano group in **4** afforded to the primary amide **6**.

The synthesis of compounds **5**, **7**, **11**, and **13** was completed from the corresponding disubstituted cyclohexanones **19** (Scheme 3). Protection of **19** with ethylene glycol, followed by partial reduction of the cyano group with $LiAlH_4$ gave aldehyde **33**. Reaction of **33** with diethyl cyanomethylphosphonate and sodium hydride produced unsaturated nitrile **34**. Hydrogenation of **34**, followed by removal of the ketal protecting group afforded ketone **35**. Reductive amination of **35** with cyclopropylamine followed by standard amide bond formation produced the nitrile **5**. Finally, hydrolysis of the cyano group to the primary amide afforded carboxamide derivatives **5**, **7**, **11**, and **13**.

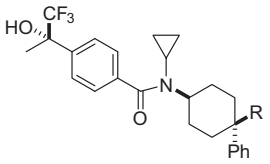
Syntheses of compounds **8** and **10** were accomplished in a straightforward manner, starting from the appropriate 4,4-disubstituted cyclohexanone **19** (Scheme 4). Primary amine intermediate **37** was prepared from **19**. Reductive amination of **19** with cyclopropylamine, followed by amide bond formation produced 4-phenyl-4-cyano cyclohexylbenzamide **36**, which was reduced to amine **37** in the presence of Raney nickel. The targeted compounds **8** and **10** were then synthesized via a coupling reaction of **37** with either methylsulfonyl or acetyl chloride, respectively.

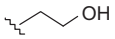
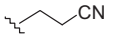
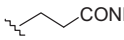
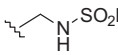
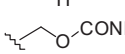
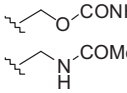
Heteroarylcarbamates **17** and **18** were prepared from cyclohexanones **38** as shown in Scheme 5. Reductive amination followed by standard amide bond formation gave intermediate **40**, which was reduced to primary alcohols. The desired *trans* alcohol **41** was separated by silica gel chromatography and treated with trichloroacetyl isocyanate as before to produce carbamates **17** and **18**.

Compounds were evaluated for the in vitro inhibition of human and mouse 11β-HSD1 enzymes, as well as in cell-based assays. 11β-HSD1 enzyme activity was determined by measuring the conversion of [^{3}H]-cortisone to [^{3}H]-cortisol. The product [^{3}H]-cortisol, captured by an anti-cortisol monoclonal antibody conjugated to scintillation proximity assay (SPA) beads, was quantified with a microscintillation plate reader. Biochemical enzyme assays were performed with baculovirus-produced recombinant full-length human 11β-HSD1 as the enzyme source and NADPH as cofactor (h-HSD1 IC_{50}). Cell-based enzyme assays (h-293 IC_{50}) utilized HEK293 cells stably expressing recombinant human full-length 11β-HSD1 as the enzyme source without supplementation of NADPH. Inhibition of 11β-HSD1 in adipocytes was evaluated by a cell-based [^{3}H]-cortisone conversion assay with differentiated human fat cells and media containing 3% of human serum albumin (HSA). IC_{50} values for enzyme inhibition were calculated with a dose response curve fitting algorithm with at least duplicate sets of samples. These compounds were also tested against the 11β-HSD2 enzyme in the SPA assay to determine 11β-HSD selectivity. All analogs had IC_{50} >10 μM against 11β-HSD2, highlighting the excellent inhibitor selectivity between the two 11β-HSD isoforms.

Our initial optimization efforts began with the goal of improving the potency of **1** by replacement of the cyclopropyl with a phenyl group and the replacement of the hydroxyl moiety with carbon tethered polar groups such as alcohols, nitriles, and primary amides (Table 1). Compounds with a one-carbon spacer between the polar group and the cyclohexane ring (**2**, **4**, and **6**) were more potent than **1** in both biochemical and cellular assays. Increasing the spacer length from one to two carbons resulted in a further potency improvement (**3**, **5**, and **7**). These observations indicated that spacer length and polarity in the features pendant from the 4-position on the cyclohexane ring were important for 11β-HSD1 inhibitory activity. Within the two-atom length sub-series,

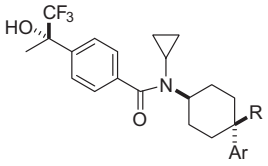
Table 1
4,4-Disubstituted cyclohexylbenzamide 11β-HSD1 inhibitors

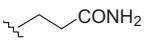
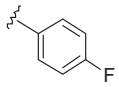
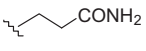
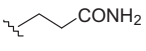
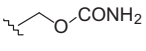
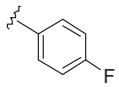
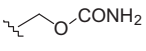
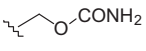
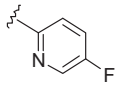
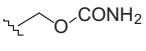
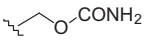


Compound	R	h-HSD1[a] SPA IC_{50} (nM)	h-293[a] IC_{50} (nM)	h-293[a] + 3% HSA IC_{50} (nM)	Adipocyte[a] + 3% HSA IC_{50} (nM)	% TO at 30 min HLM	% TO at 30 min RLM	PXR% of control at 2 μM
1		14	52	121	50	<10	<10	<5
2		2.2	41	—	—	10	32	<5
3		1.9	12	35	15	<10	<10	6
4		1.4	10.4	—	—	19	17	9
5		3.9	—	—	—	10	51	—
6		1.4	16	92	—	<10	<10	<5
7		0.8	3.9	37	17	<10	<10	<5
8		1.7	86	259	20	<10	21	<5
9		1	4.6	20	8.8	<10	<10	<5
10		2.2	6.7	129	17	<10	<10	<5

[a] IC_{50} values determined by scintillation proximity assay (SPA), and all potency data are reported as the average of at least two determinations; h-293 = HEK 293 cells stably transfected with full-length human 11β-HSD1; HSA = human serum albumin; adipocyte (3% HSA) IC_{50} values determined by cell-based [^{3}H]-cortisone conversion assay with differentiated human fat cells and media containing 3% human serum albumin; HLM and RLM = human and rat liver microsomes, respectively; %TO = % turnover.

Table 2
4,4-Disubstituted cyclohexylbenzamide 11β-HSD1 inhibitors



Compound	Ar	R	h-HSD1[a] SPA IC_{50} (nM)	h-293[a] IC_{50} (nM)	h-293[a] + 3% HSA IC_{50} (nM)	Adipocyte[a] + 3% HSA IC_{50} (nM)	% TO at 30 min HLM	% TO at 30 min RLM
11			1.3	7	109	36	<10	<10
12			1.8	12	55	24	<10	<10
13			9.6	—	—	>100	<10	<10
14			0.6	5.7	135	14	<10	<10
15			0.5	15	34	12	<10	<10
16			3.5	19	74	25	<10	<10
17			2.4	30	93	31	<10	<10
18			1.8	16	63	18	<10	<10

[a] IC_{50} values determined by scintillation proximity assay (SPA), and all potency data are reported as the average of at least two determinations; h-293 = HEK 293 cells stably transfected with full-length human 11β-HSD1; HSA = human serum albumin; adipocyte (3% HSA). IC_{50} values determined by cell-based [^{3}H]-cortisone conversion assay with differentiated human fat cells and media containing 3% of human serum albumin; HLM and RLM = human and rat liver microsomes, respectively; %TO = % turnover.

the methysulfonamide **8** exhibited the biggest potency shift in cell-based assays and the acetyl amide **10** suffered a significant potency decrease upon addition of human serum albumin (HSA). The carbamate **9** showed an optimal cellular potency within this set of compounds. Overall, compounds **7** and **9** showed a significant increase in potency compared with **1** (~17-fold in the biochemical assay; ~13-fold in the h-293 cell-based assay; and ~threefold in adipocyte cell-based assay, respectively). Moreover, the two compounds exhibited an optimal combination of limited PXR risk and excellent metabolic stability in human and rat liver microsomes (HLM and RLM).

Having identified the two-atom tethered primary amide and carbamate as the best functional groups with respect to activity, we then turned our attention to the modification of the phenyl group on the 4-position of the cyclohexane ring (Table 2). Within the primary amide sub-series, the 4-(2-fluorophenyl) substituted cyclohexylbenzamide **11** appeared to be potent in the biochemical assay, but showed a considerable potency shift in the cellular assay upon addition of HSA (~15-fold). On the other hand, pyridine analogue **13** exhibited a significant potency decrease in the adipocyte cell-based assay with 3% HSA. The 4-(4-fluorophenyl) **12** showed minimal potency shifts in the cellular assays with and without the addition of HSA. Compounds in the carbamate sub-series that had a heteroaryl ring in the 4-position showed a greater potency shift in the cellular assays (**16**, **17**, and **18**), and **15** constituted the optimal carbamate containing compound. All of the amides and carbamates proved to have excellent in vitro metabolic stability in both human and rat liver microsomes (HLM and RLM turnover <10%), as well as limited PXR activation.

Since compounds **9**, **14**, **16**, and **18** exhibited satisfactory potency in the adipocyte assay and in vitro metabolic stability, these compounds were examined in rat pharmacokinetic experiments (Table 3). It was found that **9**, **14**, and **16** had a low clearance (CL = 0.15–0.21 L/h/kg) and oral bioavailability (%*F*) between = 20–64%. Compound **18** had moderate clearance (CL = 0.82) and oral bioavailability (%*F* = 20). In addition, these compounds also inhibited the rat 11β-HSD1 enzyme (see Table 4, IC_{50} = 30–220 nM). We decided to test these molecules in a pharmacodynamic model in rats and assess the effects of oral administration towards inhibition of 11β-HSD1. Compounds **9**, **14**, **16**, and **18** were dosed orally in Sprague–Dawley rats at 4, 20, and 100 mg/kg, respectively. Two

Table 3
Rat pharmacokinetic data for compounds **9**, **14**, **16**, and **18**

Compound	CL (iv, L/kg/h)	V_{dss} (L/kg)	$T_{1/2}$ (iv, h)	*F* (po, %)	AUC (po, μg h/L)
9	0.16	1.0	3.9	20	2770
14	0.15	1.28	5.2	48	6790
16	0.21	1.24	4.2	64	6180
18	0.82	3.7	4.5	25	618

Dosed iv 0.5 mg/kg, po 2.0 mg/kg.

Table 4
Dose dependent inhibition of 11β-HSD1 by **9**, **14**, **16**, and **18** in the rat ex vivo PD model

Compound	h-HSD1 [a] IC_{50} (nM)	r-HSD1 [a] IC_{50} (nM)	Inhibition over vehicle (%)		
			4 (mg/kg)	20 (mg/kg)	100 (mg/kg)
9	1.0	86	23	43	71
14	0.63	144	27	43	70
16	3.5	220	14	44	72
18	1.8	30	6	38	75

[a] Values are means of at least two determinations.

hours after receiving the dose, animals were sacrificed, epididymal fat was isolated and incubated in media containing [^{3}H]-cortisone. Rat 11β-HSD1 activity was then measured by detection of [^{3}H]-cortisol. Although the inhibition of the rat enzyme was less potent than the inhibition of the human enzyme, a dose-dependent reduction in cortisol production was observed for all of four compounds (Table 4).

Furthermore, a non-human primate pharmacodynamic model was used to evaluate the effects of the compound toward inhibition of 11β-HSD1. Since compound **7** had suitable pharmacokinetic profile in cynomologous monkey (CL = 0.06 L/h/kg, %*F* = 81%) and activity against cynomologous monkey 11β-HSD1 enzyme (IC_{50} = 22 nM), this molecule was selected for evaluation in our non-human primate ex vivo pharmacodynamic model. In this study, non-human primates were dosed orally with 4, 20, and 100 mg/kg of inhibitor **7**. At 2 h post-dose, mesenteric fat tissues were collected. Following 1 h incubation of these tissues in media containing [^{3}H]-cortisone, 11β-HSD1 activity was measured through detection of tritiated cortisol levels using a scintillation proximity assay. Relative to controls, all dose groups showed a decrease in [^{3}H]-cortisol production in mesenteric fat (Fig. 2). These results demonstrate that **7** is effective in lowering 11β-HSD1 adipose activity in cynomolgus monkeys when administered orally and has a reasonable potential to serve as an effective orally bioavailability inhibitor of 11β-HSD1 in humans (Table 5).

Finally, the co-crystal structure of **7** with human 11β-HSD1 containing NADP cofactor was obtained by X-ray crystallography (Fig. 3).[35] As was seen with our earlier published structures from this and closely related compound classes,[30–32] **7** binds to the substrate site in a V-shape with its trifluoromethylcarbinol group pointing toward the cofactor $NADP^+$ side. Both the central and the primary amide carbonyl groups have hydrogen bond interactions with the adjacent protein molecules.

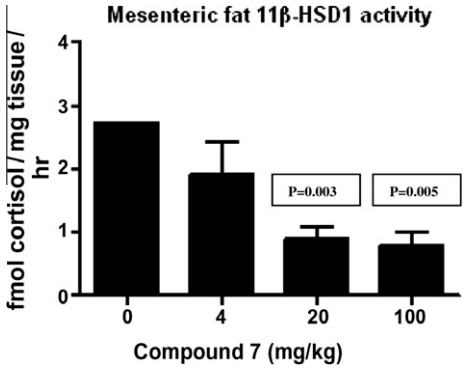


Figure 2. Ex vivo 11β-HSD1 enzyme activity in intact mesenteric fat collected from cynomolgus monkeys dosed orally with compound **7**. Plasma and mesenteric fat samples were collected 2 h after compound was administered orally in dosing vehicle (1% methylcellulose and 1% Tween 80 in sterile water). 11β-HSD1 enzyme activity was measured as the [^{3}H]-cortisol formed after 1 h incubation of fat samples in reaction buffer containing [^{3}H]-cortisone at 37 °C. Compound concentration in plasma was determined by LC/MS/MS.

Table 5
Plasma exposure in cynomolgus monkeys after oral dosing with **7**

PO dose	*N*	[**7**] (ng/mL) plasma
Vehicle	6	BQL
4 mg/kg	5	151 ± 93
20 mg/kg	6	1999 ± 2144
100 mg/kg	5	1709 ± 779

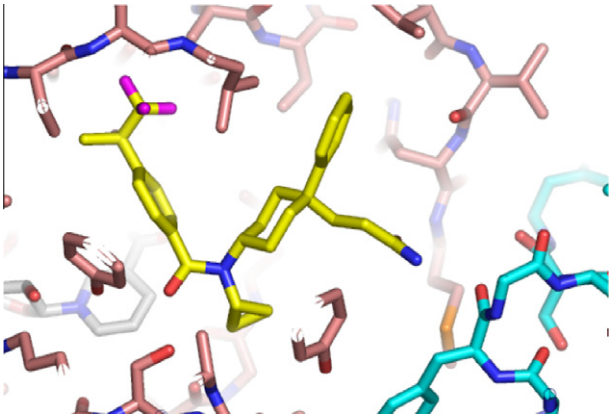


Figure 3. Co-crystal structure of compound **7** in human 11β-HSD1. The protein is shown in both stick and molecular surface representations which are color coded (red for oxygen atoms, blue for nitrogen, orange for sulfur, and pink and cyan for carbon). The inhibitor and the cofactor $NADP^+$ are shown in sticks and color coded gray for carbon atoms in $NADP^+$ and yellow for the inhibitor, the fluorine atoms of the inhibitor are colored in magenta.

In summary, modification of compound **1** at the 4-position of the cyclohexane ring led to the discovery of the two-carbon tethered primary amide and carbamate groups, producing significant improvements in biochemical and cellular potency. Furthermore, these modifications resulted in a group of potent, selective, and orally bioavailable compounds that demonstrated 11β-HSD1 inhibition in both rat and cynomolgus monkey ex vivo models. Finally, X-ray co-crystallographic data of **7** with 11β-HSD1 revealed the key small molecule–protein complex interactions for the binding of this class of 11β-HSD1 inhibitor.

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