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Discovery and optimization of piperidyl benzamide derivatives as a novel class of 11β-HSD1 inhibitors

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ABSTRACT

Discovery and optimization of a piperidyl benzamide series of 11β-HSD1 inhibitors is described. This series was derived from a cyclohexyl benzamide lead structures to address PXR selectivity, high non-specific protein binding, poor solubility, limited in vivo exposure, and in vitro cytotoxicity issues observed with the cyclohexyl benzamide structures. These efforts led to the discovery of piperidyl benzamide **15** which features improved properties over the cyclohexyl benzamide derivatives.

One of the key regulatory roles of glucocorticoids is glucose homeostasis. In this regard, glucocorticoids regulate gluconeogenesis in the liver and inhibit peripheral glucose uptake in muscle and adipose tissue. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), an NADPH-dependent reductase, is a key enzyme that converts the inactive glucocorticoid cortisone to the active glucocorticoid hormone cortisol in liver, adipose, and brain tissues. Excessive concentrations of glucocorticoids in the liver and adipose tissue can lead to glucose intolerance and insulin resistance. Therefore, selective inhibition of the 11β-HSD1 enzyme has been considered as a promising strategy to improve insulin sensitivity and treat type II diabetes.[1,2]

Previously we reported on a series of cyclohexyl benzamide derivatives, exemplified by compound **1**, as potent 11β-HSD1 inhibitors (Fig. 1).[3] Compound **1** exhibited excellent in vitro inhibition of human 11β-HSD1 enzyme and favorable pharmacokinetic profiles in the rat, cynomolgus monkey, and dog. Furthermore, compound **1** showed a dose-dependent decrease in adipose 11β-HSD1 activity in a monkey ex vivo pharmacodynamic model.[3]

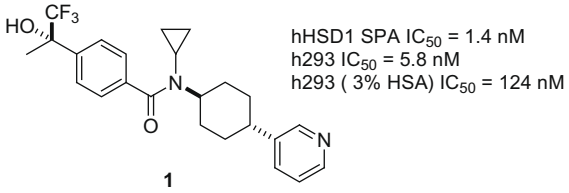

Figure 1. Representative cyclohexyl benzamide 11β-HSD1 inhibitor.[3,8]

However, **1** exhibited low micromolar range in vitro cytotoxicity in HeLa cells (IC_{50} = 2.5 μM).[4]

The X-ray co-crystal structure of **1** with the 11β-HSD1 enzyme showed that the pyridyl ring points toward the solvent exposed region of the protein dimer interface and does not make any discernable specific interaction with the active site of the protein.[3] Combined with our initial SAR,[3] this gave some indication that the core cyclohexyl benzamide motif was the essential binding component of these molecules. We hypothesized that, by replacing the pyridyl group with other functional groups, we could modulate physicochemical properties without disrupting critical interactions with the enzyme. Moreover, in addition to enhancing the structural diversity of compounds at the 4-position of the cyclohexane ring,

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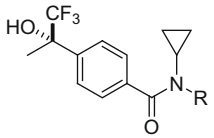
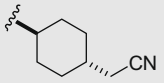
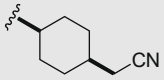
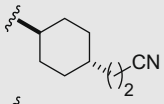
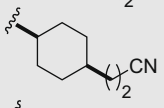
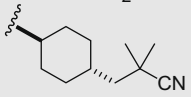
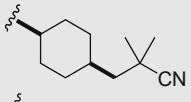
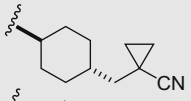
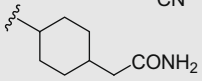
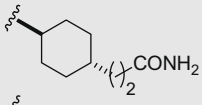
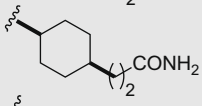
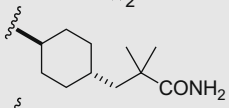
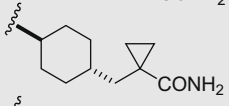
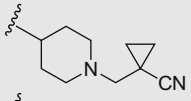
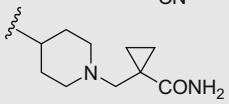
Table 1
SAR of cyclohexyl and piperidyl benzamides with polar alkyl susbstituents



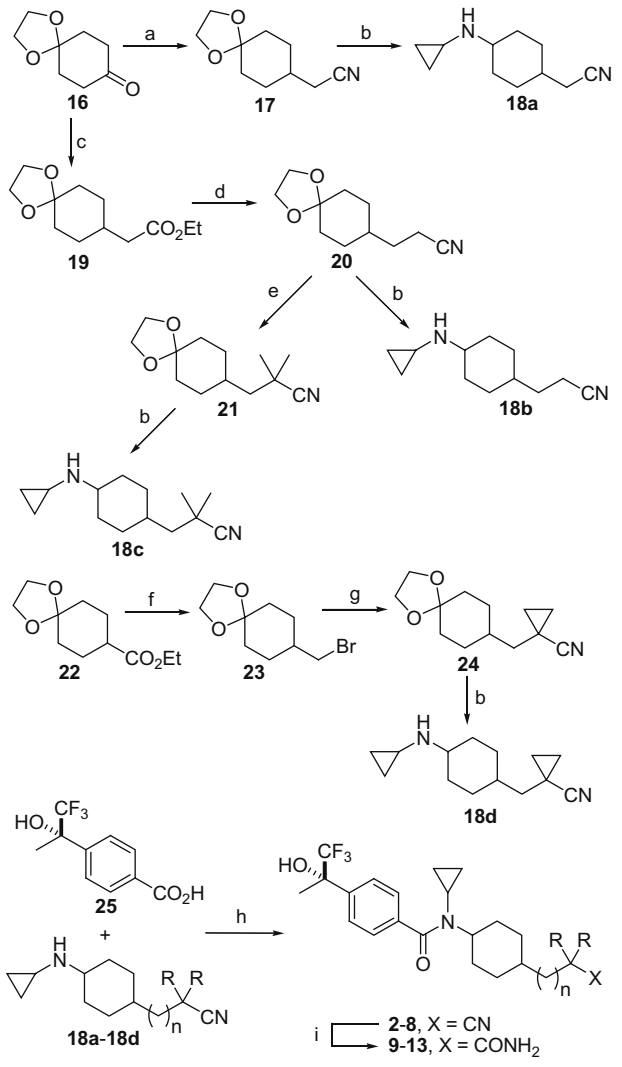
Compound[a]	R	hHSD1 SPA[b] IC_{50} (nM)	h293[c] (cell) IC_{50} (nM)	h293 (cell, 3% HSA[d]) IC_{50} (nM)	% Remaining z@ 30 min		PXR[g] activation % of control[h] @ 2 μM	HeLa cytotoxicity IC_{50} (μM)
					hLM[e]	rLM[f]		
2		5.5	16	60	>90	73	75	N/D[i]
3		7.6	52	202	70	5	74	N/D
4		0.7	1.4	11	>90	42	30	>10
5		2.8	10	130	64	<5	56	>10
6		0.88	1.4	30	>90	77	60	N/D
7		2.0	8	129	N/D	N/D	N/D	N/D
8		0.6	1.2	28	>90	>90	60	4
9	 (trans/cis =1/1)	36	315	1190	N/D	N/D	1	N/D
10		2.4	17	77	>90	>90	5	>10
11		11	109	417	>90	72	100	>10
12		3.8	6.1	97	>90	>90	4	>10
13		1.7	5.0	90	>90	>90	6.5	8
14		11	56	108	86	28	3	>10
15		14	167	148	>90	86	4	>10

[b,c,d]All potency data are reported as the average of at least two determinations.
[a] All compounds gave satisfactory ^1H NMR, HPLC, and MS data in full agreement with their proposed structures, and purity (>95%) was determined by HPLC analysis.
[b] IC_{50} values determined by scintillation proximity assay.
[c] h293 = HEK 293 cells stably transfected with full-length human 11β-HSD1.
[d] HSA = human serum albumin.
[e] hLM = human liver microsomes.
[f] rLM = rat liver microsomes.
[g] PXR = pregnane X receptor.
[h] Rifampin (@ 10 μM) used as a positive control.
[i] N/D = not determined.

such modifications would potentially offer an opportunity to address the off-target cytotoxicity observed in HeLa cells without affecting the 11β-HSD1 activity.

We felt that extending polar groups from the 4-position of the cyclohexyl ring would enhance the solubility of the compounds. We therefore initiated our efforts by the systematic introduction of polarity via carbon tethered nitrile and primary amide groups (compounds **2**–**13** in Table 1).

Compounds **2**–**13** were synthesized via the routes outlined in Scheme 1. Three cyclohexylamine intermediates (**18a**–**18c**) were prepared from the monoacetal (**16**). Reaction of diethyl cyanomethylphosphonate sodium salt with **16**, followed by hydrogenation afforded nitrile **17**.[5] In a similar manner, **16** was transformed into ester **19,** which was followed by carbon-chain elongation of **19** to provide homologated nitrile **20**.[6] α,α-Dimethylnitrile **21** was prepared via sequential methylation of **20**. The corresponding cyclopropyl analog **24** was synthesized by conversion of ester **22** into bromide **23** followed by alkylation of **23** with the cyclopropanecarbonitrile anion.[7] All these protected ketone intermediates (**17**, **20**, **21**, and **24**) were converted into amines **18a**–**18d** by removal of the ketal protecting group, followed by reductive amination with cyclopropylamine. Finally, the target 4-cyanoalkyl-cyclohexyl benzamides, **2**–**8** were synthesized via amide coupling reaction of these amines with chiral acid **25**[3] followed by separation of each geometric isomer (cis and trans) using silica-gel column chromatography. Further hydrolysis of the cyano group to the primary amide provided carboxamide derivatives, **9**–**13**.



Scheme 1. Reagents and conditions: (a) i—$(EtO)_2P(O)CH_2CN$, NaH, DMPU, THF, 95%; ii—10% Pd/C, H_2, AcOH/H_2O, 95%; (b) i—a 1:2 (v/v) mixture of 3 N aqueous HCl and THF, quantitatively; (ii) cyclopropylamine, $NaBH(OAc)_3$, AcOH, 1,2-DCE, 80–95%; (c) i—$(EtO)_2P(O)CH_2CO_2Me$, NaH, DMPU, THF, 85%; ii—10% Pd/C, H_2, EtOAc, 98%; (d) i—$LiAlH_4$, ether, 91%; ii—MsCl, TEA, DCM, quantitatively; iii—NaCN, DMSO, 71%; (e) i—LDA, MeI, THF, 76%; ii—LDA, MeI, THF, 70%; (f) i—$NaBH_4$, 10% MeOH-DME, 77%; ii—CBr_4, Ph_3P, benzene, 81%; (g) LDA, cyclopropanecarbonitrile, THF, −78 °C, 63%; (h) HBTU, HOBt, DIEA, DMF, 30–60% (cis/trans = 1/1–7.8/1); i—KOH, *t*-BuOH, 85 °C, 48–81%.

All synthesized compounds were evaluated for the inhibition of human and mouse 11β-HSD1 enzymes, as well as in cell-based assays.[8] Consistent with our previous publication,[3] trans isomers (**2**, **4**, **6**, **10**) were typically more potent than *cis* isomers (**3**, **5**, **7**, **11**) in both biochemical and cellular assays. A 2-carbon spacer length between the polar functional group (-CN or $-CONH_2$) and the cyclohexane ring resulted in a substantial increase in potency relative to the 1-carbon spacer (**2**, **3** vs **4**, **5**). The improvement in biochemical potency for **4** and **5** may reflect a greater tolerance of polar features distanced further towards solvent exposed regions of the protein. Within the 2-carbon linked nitrile and primary amide molecules, both biochemical potency and cellular potency were slightly decreased in primary amides (**10**, **11**, **12**, **13**) relative to the corresponding nitriles (**4**, **5**, **6**, **8**). Also, compounds in this series showed 5- to 20-fold shift in their IC_{50} values in the h293 cell-based assays upon addition of HSA.

As observed previously in mono-substituted cyclohexyl benzamides,[3] trans isomers were found to be more metabolically stable than cis isomers in both human liver microsomes and rat liver microsomes (**2**, **4**, **10** vs **3**, **5**, **11**). Replacement of the nitrile group with a primary amide improved the rat in vitro metabolic stability significantly (**4**, **6** vs **10**, **12**), while installation of α,α-disubsituents such as *gem*-dimethyl or cyclopropyl group further enhanced in vitro metabolic stability in rat (**4** vs **6**, **8**).

Compounds were also evaluated for their potential to activate PXR, a liability that can potentially lead to upregulation of CYP3A4.[9] In most cases, trans isomers exhibited reduced PXR activity in vitro (**4**, **10** vs **5**, **11**), while conversion of the nitrile group to the more polar primary amide group in trans isomers significantly reduced PXR activation potential (**2**, **4**, **6**, **8** vs **9**, **10**, **12**, **13**).

Of significance was the observation that most compounds evaluated had marked reduction in cytotoxicity ($IC_{50} \geqslant 10\ \mu M$) relative to **1**. Moreover, compounds **10** and **12** showed optimal combination of limited cytotoxicity and PXR potential, good potency and microsomal stability and were therefore selected for rat pharmacokinetic (PK) studies.[10a,10b] However, it was found that the in vitro rat liver microsomal stability of **10** and **12** (>90% remaining after 30 min for both **10** and **12**) was not predictive of the in vivo rat clearance. Compounds **10** and **12** were found to have high and moderate clearance in rat [CL (iv) = 3.0 and 1.3 L/h/kg, respectively] in addition to moderate oral bioavailability (%F = 22 and 32, respectively).

Metabolic studies utilizing compound **10** with human and rat hepatocytes revealed that hydrolysis of the primary amide to the corresponding carboxylic acid occurred, followed by glucuronidation to **27** (Fig. 2). This observation provided an explanation for the disconnect between the observed in vitro microsomal stability and the rat in vivo clearance. Introduction of α,α-dialkyl substituents improved in vitro stability in both human and rat hepatocytes (**10** vs **12**). Moreover, replacement of the *gem*-dimethyl group with a cyclopropyl group significantly enhanced the in vitro hepatocyte stability in human and rat (Table 2). This superior stability of the cyclopropyl compound **13** relative to the *gem*-dimethyl compound **12** can potentially be explained by a combination of steric and electronic effects.[11]

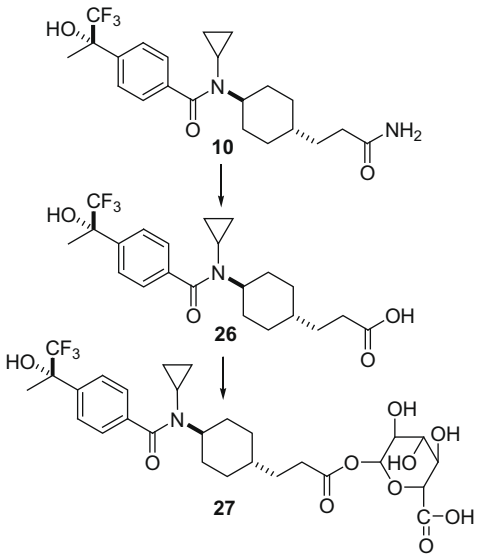
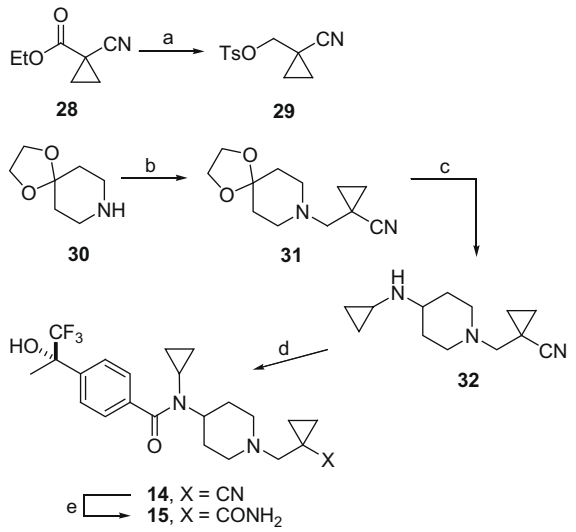


Figure 2. Metabolism of compound **10** in human and rat hepatocytes.

Table 2
In vitro hepatocyte stability of compounds **10**, **12**, and **13**

Compound	Hepatocyte stability % remaining @ 90 min	
	hHep[a]	rHep[b]
10	32	<5
12	84	10
13	>90	73

[a] hHep = human hepatocytes.
[b] rHep = rat hepatocytes.



Scheme 2. Reagents and conditions: (a) i—$NaBH_4$, 10% MeOH–DME, 90%; ii—TsCl, pyridine, 0 °C, 75%; (b) **29**, DIEA, AcCN, 85 °C, 80%; (c) (i) 5% H_2O–TFA, 88%; ii—cyclopropylamine, $NaBH(OAc)_3$, AcOH, 1,2-DCE, 100%; (d) **25**, EDCI, HOAt, $NaHCO_3$, DMF, 74%; (e) KOH, *t*-BuOH, 85 °C, 45%.

Having identified a pendant feature that appeared well tolerated and stable in hepatocytes, an effort was made to further enhance solubility and stereosimplicity by optimization of the cyclopropylamide in the context of a more polar piperidine ring architecture.

Piperidyl benzamide derivatives, **14** and **15** were synthesized in a straightforward manner, through a sequence illustrated in Scheme 2. Ethyl 1-cyanocyclopropanecarboxylate **28** was prepared according to the literature procedures[12] and was converted into tosylate **29** following ester reduction. *N*-Alkylation of piperidine **30** with **29** provided **31**. Ketal hydrolysis followed by reductive amination afforded amine intermediate **32**. Amide coupling of **32** with chiral acid **25** yielded *N*-cyanoalkylpiperidyl benzamide **14**, which was transformed into the primary amide derivative **15**.

The potency and biological data of compound **14** and **15** are summarized in Table 1. Both **14** and **15** were less potent than the corresponding cyclohexyl benzamides in both biochemical and cellular assays (**14**, **15** vs **8**, **13**). However, piperidyl benzamides showed a much lower cellular potency shift upon addition of HSA (1- to 2-fold shift) compared to cyclohexyl benzamides and their cellular potency in the presence of 3% HSA was comparable to that of **13**. Plasma protein binding data for compound **13** and **15** are also consistent with the cellular potency shift upon addition of HSA (Table 3).

Whereas **14** showed a compromised in vitro metabolic stability in rats, the cyclopropyl amide **15** had improved metabolic stability. Compound **15** was evaluated in rat pharmacokinetic studies. Compound **15** exhibited low plasma clearance [CL (iv) = 0.89 L/h/kg] and excellent oral bioavailability (%F = 69).[10c] As with the cyclohexyl benzamide derivative, the in vitro human and rat hepatocyte stability assay results were in agreement with the in vivo plasma clearance of compound **15** (>90% remaining after 90 min in hHep, 69% remaining after 90 min in rHep).

The structure of compound **15** bound to human 11β-HSD1 was determined by X-ray crystallography (Fig. 3) to a resolution of 2.3 Å.[13] The co-crystal structure showed that the inhibitor binds to the substrate site in a V-shape with its trifluoromehtylcarbinol

Table 3
Plasma protein binding for compounds **13** and **15**

Compound	fu in human plasma[a]	fu in rat plasma[a]
13	0.12	0.048
15	0.48	0.20

[a] Samples analyzed on API-4000 and ultracentrifugation protocol-4 h. fu = fraction unbound.

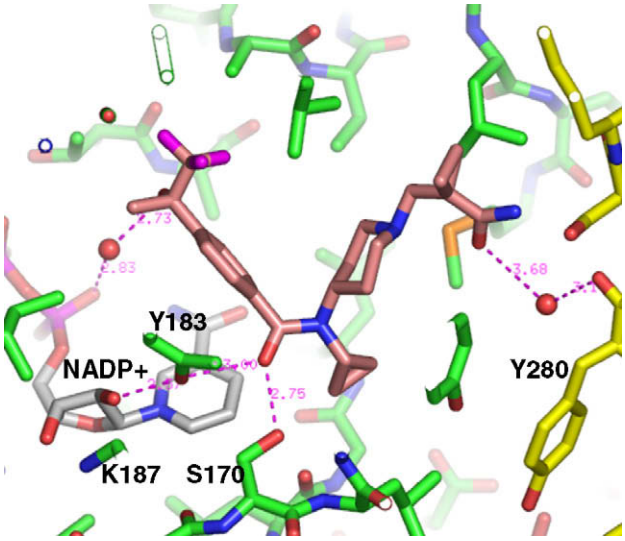


Figure 3. X-ray co-crystal structure of compound **15** in human 11β-HSD1. The key hydrogen bonding interaction of **15** to the enzyme is shown with magenta dashed line. The color scheme is red for oxygen atoms, blue for nitrogen atoms, orange for sulfur atoms, magenta for fluorine atoms. Carbon atoms are in green for protein 1, yellow for protein 2, gray for $NADP^+$, and beige for inhibitor. The water molecules are shown in red spheres.

group pointing towards the cofactor NADP + side. The central amide carbonyl group makes a hydrogen bond interaction with the hydroxyl group of S170, one of the three residues defined as the catalytic triad for 11β-HSD1 activity.[14] The cyclopropanecarboxamide group locates in the dimer interface and forms a weak water-mediated hydrogen bond with C-terminal region of adjacent protein.

In summary, we have described our efforts towards the expansion of structural diversity and optimization of a structurally novel cyclohexyl benzamide series of 11β-HSD1 inhibitors. Improvements in metabolic stability and reduction in cytotoxicity within the cyclohexyl benzamide series transferred well to the piperidyl subclass culminating in compound **15** which represents a potent and promising benzamide subclass. Compound **15** effectively addresses PXR activation, high non-specific protein binding, poor pharmacokinetic properties, and in vitro cytotoxicity issues previously observed with the cyclohexyl benzamide structures. This represents a promising step forward in our continued development of our benzamide class of 11β-HSD1 inhibitors.

References and notes

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9. Pregnane X Receptor (PXR) assay: HepG2 cells transfected with a luciferase reporter construct driven by CYP3A4 gene and human PXR cDNA. Cells were exposed to test article and the luciferase activity determined by chemilum. Rifampin is used as control.
10. a Compound **10**: dosed iv 0.51 mg/kg, po 2.0 mg/kg.; b Compound **12**: dosed iv 0.39 mg/kg, po 2.0 mg/kg.; c Compound **15**: dosed iv 0.50 mg/kg, po 2.0 mg/kg.
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