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Synthesis and evaluation of piperidine urea derivatives as efficacious 11β-hydroxysteroid dehydrogenase type 1 inhibitors in diabetic ob/ob mice

Liming Zhang, Junhua Chen, Mengmeng Ning, Qingan Zou, Ying Leng *, Jianhua Shen *

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China

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ABSTRACT

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) has attracted considerable attention as a potential target for the treatment of diabetes and metabolic syndrome. Herein we report the design, synthesis and efficacy evaluation of novel amide and urea 11β-HSD1 inhibitors. Structure–activity relationship studies led to the identification of **10c**, which was efficacious in a diabetic ob/ob mouse model and reduced fasting and non-fasting blood glucose levels after ip dosing.

Glucocorticoids are steroid hormones and play a key role in regulating glucose and lipid homeostasis.[1] Excessive levels of glucocorticoids can cause metabolic complications, including insulin resistance, central obesity, hyperglycemia, dyslipidemia, hypertension, and type 2 diabetes.[2–4] Glucocorticoid action depends on circulating hormone levels and local activation or regeneration by key enzymes, 11β-hydroxysteroid dehydrogenase isozymes (11β-HSDs).

There are two types of 11β-HSDs, 11β-HSD1 and 11β-HSD2. 11β-HSD1 functions predominantly as an NADPH-dependent reductase and converts inactive cortisone into the receptor-active glucocorticoid cortisol in humans. Moreover, it is highly expressed in the liver and adipose tissues. Thus, it locally amplifies the glucocorticoid action in specific tissues.[5,6] 11β-HSD2, on the other hand, is a NAD-dependent enzyme that catalyzes the dehydrogenase reaction of converting cortisol to cortisone (Fig. 1) and is mainly located in kidney. Inhibition of 11β-HSD1 has the potential to control cortisol concentrations in the liver and adipose without affecting systemic circulating concentrations.

11β-HSD1 deficient mice show enhanced glucose tolerance, attenuated gluconeogenic responses and improved lipid and lipoprotein profiles.[7] Conversely, transgenic mice overexpressing 11β-HSD1 selectively in adipose develop many of the features of the metabolic syndrome such as glucose intolerance, insulin resistance, dyslipidemia and hypertension.[8,9] These mouse genetic models demonstrated the connection between 11β-HSD1 and metabolic syndrome as well as diabetes. As a matter of fact, a number of small molecule inhibitors of 11β-HSD1 have been disclosed in the past few years.[10–12] In a phase 2 study, Incyte's small molecule inhibitor INCB13739 showed a positive trend in reducing fasting glucose and HbA1c with a 200 mg dose.[13] MK-0916 modestly improved HbA1c, body weight, and blood pressure in a phase 2 trial in patients with type 2 diabetes.[14]

These promising studies strongly suggest that 11β-HSD1 is a potential therapeutic target for a broad range of disorders that could be improved by decreased intracellular glucocorticoid levels (i.e., cortisol), including metabolic syndrome as well as type 2 diabetes.

Recently, we have reported that (phenylsulfonamidomethyl) benzamides and (phenylsulfonamidomethyl)nicotinamides were

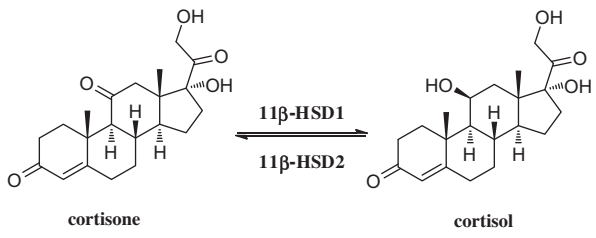

Figure 1. Interconversion of cortisone and cortisol mediated by 11β-HSD1 and 11β-HSD2.

* Corresponding authors. Tel.: +86 21 20231969; fax: +86 21 20231971 (J.S.); tel./fax: +86 21 50806059 (Y.L.).
E-mail addresses: yleng@mail.shcnc.ac.cn (Y. Leng), jhshen@mail.shcnc.ac.cn (J. Shen).

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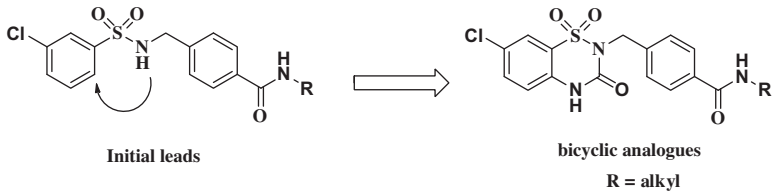


Figure 2. Strategic approach from (phenylsulfonamidomethyl)benzamides to bicyclic analogues.

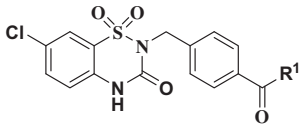
potent and selective inhibitors of 11β-HSD1.[15,16] Molecular modeling experiments demonstrated that the *N*-cycloalkyl amide moiety was the essential binding part of these molecules.[16] Preliminary structure–activity relationship (SAR) studies showed that alkyl substitution on the nitrogen of sulfonamide is beneficial to improve potency. We presumed that the enhanced potency might be influenced by the steric effect of the substituent. Given the above observations, we hypothesized the possibility of constraining the sulfonamide moiety into a six-membered ring to form bicyclic analogues (Fig. 2). We aimed to improve biological potency and increase structural novelty by incorporating a suitable bicyclic moiety. As our initial leads were generally highly lipophilic ($c\log P$ >5),[15] we also hoped to reduce the lipophilicity of these molecules to values considered more suitable for orally administered drugs. Herein we report the discovery of potent 11β-HSD1 amide and urea inhibitors with good in vivo efficacy in diabetic ob/ob mice.

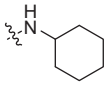
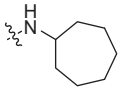
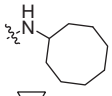
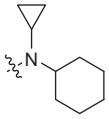
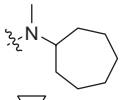
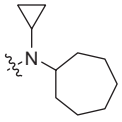
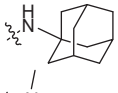
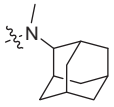
The synthesis of compound **5** is depicted in Scheme 1. Compound **1** was alkylated with methyl 4-(bromomethyl)benzoate **2** to give **3**, which was subsequently hydrolysed to yield carboxylic acid **4**. Finally, compounds **5** were obtained by coupling of various amines with the acid **4** using standard methods for amide formation.[17]

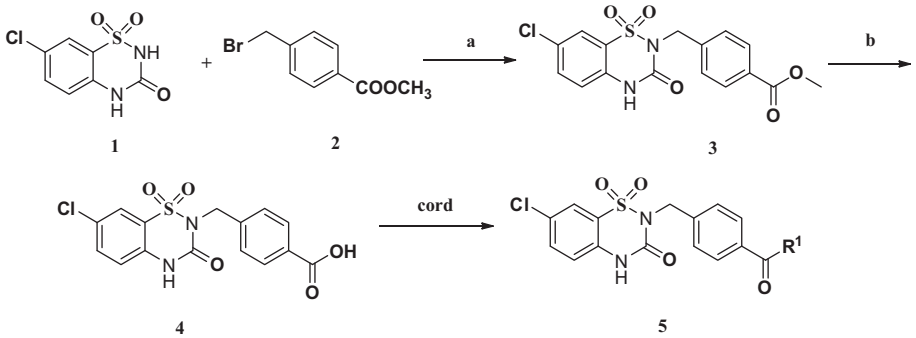
Compounds were evaluated for inhibition of human and mouse 11β-HSD1 enzymes by the scintillation proximity assay (SPA)[18] and selected compounds were evaluated in human and mouse 11β-HSD2 assays (for details, see Supplementary data). Glycyrrhizic acid and carbenoxolone were used as reference compounds.

First, the effect of modifying alkyl substitution in the amide region of compounds **5** was investigated and the results were shown in Table 1. Generally, 11β-HSD1 inhibition activity was maintained after the incorporation of a thiadiazine ring. On the distal alkyl groups, various lipophilic amides were well tolerated. The *N*-cyclohexyl amide **5a** showed a moderate potency with a human IC_{50} of 686 nM. As compared to **5a**, introduction of cycloheptyl (**5b**) or cyclooctyl (**5c**) only resulted in improvement of potency in mouse assays. When *N*-cyclohexyl amide (**5a**) was replaced with the more lipophilic 1-adamantanyl amide group (**5g**), the potency increased in both human and mouse enzymatic assays. Introduction of alkyl

Table 1
11β-HSD1 inhibition for 4-substituted benzamides **5**

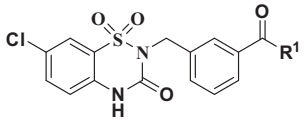


Compound	R^1	11β-HSD1 IC_{50} (nM)	
		Human	Mouse
5a		686	529
5b		802	145
5c		443	180
5d		60	185
5e		90	210
5f		55	84
5g		47	14
5h		70	55
Glycyrrhizic acid		10	8



Scheme 1. Synthesis of 4-substituted benzamides **5**. Reagents and conditions: (a) K_2CO_3, DMF, rt; (b) 1 mol/L NaOH, MeOH, reflux; (c) (i) $SOCl_2$, CH_2Cl_2, reflux; (ii) Et_3N, amine, CH_2Cl_2; (d) EDCI, HOBt, CH_2Cl_2, amine.

Table 2
11β-HSD1 inhibition for 3-substituted benzamides **6**



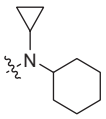
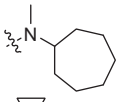
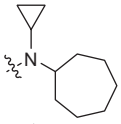
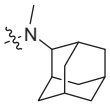
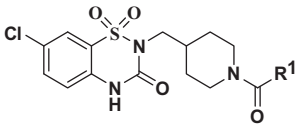
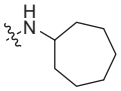
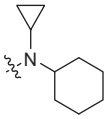
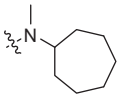
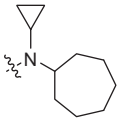
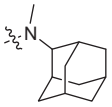
Compound	R^1	11β-HSD1 IC_{50} (nM)	
		Human	Mouse
6a		3	58
6b		4	401
6c		1	258
6d		4	69
Glycyrrhizic acid		10	8

Table 3
11β-HSD1 inhibition for urea analogues **10**



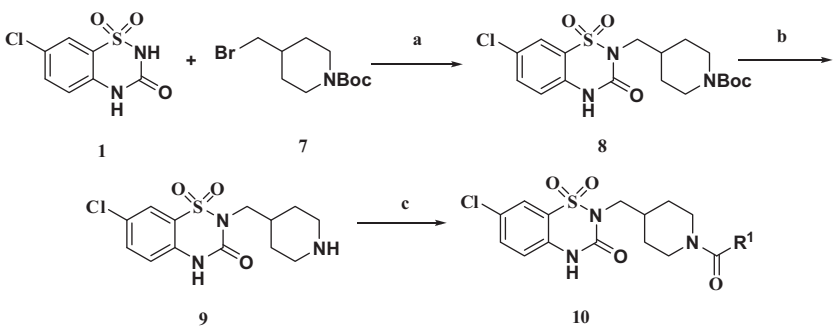
Compound	R^1	11β-HSD1 IC_{50} (nM)	
		Human	Mouse
10a		283	17
10b		121	37
10c		21	0.41
10d		517	10
10e		158	2
Glycyrrhizic acid		10	8

substituent at the nitrogen of the amide proved to be beneficial for enzyme activity (**5a**, **5b** vs **5d**, **5f** respectively). Compound **5d** showed 11-fold improvement in potency against human 11β-HSD1 when compared to **5a**. Compound **5g** which contained a 1-adamantanyl amide substituent exhibited the greatest potency in this series, (human-1 IC_{50} = 47 nM, mouse-1 IC_{50} = 14 nM) and was 14-fold more potent than **5a**. These results suggest that large lipophilic amides could better accommodate the hydrophobic space in the enzyme binding pocket, so these groups were kept in further optimization.

Next, 3-substituted benzamide analogues **6** were prepared to determine the effect of amide location on 11β-HSD1 potency. The synthesis of those analogues was similar to **5** by replacement of intermediate **2** with methyl 3-(bromomethyl)benzoate. The inhibitory activities of compounds **6** against 11β-HSD1 are compiled in Table 2. In accord with previous studies,[19] 'V-shaped' isomers **6** were more potent than linear amide **5** in human assays. All compounds of **6** showed high binding affinities to human 11β-HSD1 enzyme (human IC_{50} <5 nM). However, change of amide location was found to be detrimental to potency in mouse assays, and the mouse IC_{50} of these analogues were 20- to 260-fold lower. Such discrepancies in 11β-HSD1 activity between species might be anticipated, because the mouse shares only 79% homology with the human enzyme.[20] However, different species activity presents a challenge for identifying predictive in vivo models.

To narrow species discrepancies, we then turned our attention to the central phenyl region of the molecule. We replaced the phenyl ring with piperidine and synthesized piperidine-1-carboxamide analogues **10**. The synthesis of **10** was summarized in Scheme 2. Compound **8** was prepared by alkylation reaction between **1** and **7** in the presence of NaH in DMF. Conversion of **8** to **9** was achieved by removing the Boc group via TFA. Finally, treatment of intermediate **9** with triphosgene followed by corresponding amines generated the target analogues **10**.[17]

Table 3 displays limited examples from SAR study of **10**. Replacement of the phenyl ring with piperidine led to improved potency against mouse 11β-HSD1, but reduced potency against



Scheme 2. Synthesis of piperidine ureas **10**. Reagents and conditions: (a) NaH, DMF, 60 °C; (b) TFA, 1,4-dioxane, 60 °C; (c) triphosgene, Et_3N, amine, CH_2Cl_2.

Table 4
11β-HSD1 and 11β-HSD2 inhibition for compound **10**

Compounds	11β-HSD1 IC_{50} (nM)		11β-HSD2 IC_{50} (nM)	
	Human	Mouse	Human	Mouse
10b	121	37	2200	>100,000
10c	21	0.41	860	>100,000
10e	158	2	720	>100,000
Glycyrrhizic acid	10	8	1	
Carbenoxolone				82

Table 5
Pharmacokinetic profiles for **6c** and **10c** in male Sprague–Dawley rats[a]

Compounds	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-t} (ng h/mL)	$AUC_{0-\infty}$ (ng h/mL)	MRT (h)	$t_{1/2}$ (h)
6c	2.3	3.86	15.5	16.1	4.02	2.02
10c	2.0	46.8	173	209	4.24	2.30

[a] Mean values ($n = 3$) from three rats for oral dosing.

the human enzyme. The mouse IC_{50} of **10** is 3- to 80-fold higher than human IC_{50}. Compound **10c** was the best inhibitor among this series and exhibited a human 11β-HSD1 IC_{50} of 21 nM. This series of analogues demonstrated that the phenyl ring was not essential for 11β-HSD1 potency and could be replaced with more flexible ring.

Lipophilicity is a key physicochemical property for drug candidates.[21] According to generally accepted criteria of drug-likeliness, the calculated log*P* (*c*log*P*) value of a drug candidate should be smaller than five.[22,23] As hoped, introduction of a bicyclic ring had a major impact on the overall lipophilicity. Compared with our initial leads, compounds **5**, **6** and **10** demonstrated favorable *c*log*P* profiles (*c*log*P* <5),[24] suggesting that they might have improved drug-like properties.

11β-HSD2 is an NAD-dependent dehydrogenase that catalyzes the reverse reaction of 11β-HSD1 to inactivate cortisol to cortisone. Inhibition of 11β-HSD2 might cause hypokalemia and hypertension due to kidney mineralocorticoid receptor activation.[1,25] Therefore, 11β-HSD1 inhibitors would need to demonstrate selectivity over 11β-HSD2 to avoid undesirable side effects. Selected compounds of **5** and **6** exhibited high selectivity against human 11β-HSD2 with IC_{50} selectivity ratio >1000. Many of the compounds **10** exhibited marginal selectivity and left an area for further improvement (Table 4).

Based on these SAR studies, compound **6c** and **10c** were selected for pharmacokinetic evaluation (Table 5). Unfortunately both compounds exhibited poor pharmacokinetics in rodent models upon oral dosing. As an example, compound **10c** produced poor plasma exposures upon dosing in rats at a 5 mg/kg oral dose with an AUC of 147 ng h/mL. Because of the poor rat pharmacokinetic profiles, **10c** was dosed by intraperitoneal injection for in vivo pharmacodynamic evaluation.

The in vivo efficacy of compound **10c** was investigated in ob/ob mice, an animal model of type 2 diabetes. As shown in Figure 3, after 5 days of the treatment with compound **10c** (50 mg/kg) once daily by intraperitoneal injection, the fasting blood glucose level in ob/ob mice was reduced by 37.1%, compared with that in vehicle control mice. The fasting blood glucose level in ob/ob mice was maintained the reduction by the rate 33.3%, 39.4%, and 36.1% at days 10, 15, and 20, respectively (Fig. 3a), which reflected the stable performance of compound **10c** on glucose control in ob/ob mice. Similarly, the non-fasting blood glucose level in ob/ob mice was reduced by 25% after 5 days treatment with compound **10c**. Moreover, the effect of compound **10c** in lowering the blood glucose level in ob/ob mice was also sustained at the rate of 27.9%, 34.6%, and 35.6% at day 10, 15, and 20, respectively (Fig. 3b). During the 20 days treatment, compound **10c** reduced the fasting and non-fasting blood glucose level in ob/ob mice by an average rate of 31.4% and 25.7%, respectively (Fig. 3a and b). In our studies, the effects of stress response on fasting and non-fasting glucose levels were not observed (for details, see Supplementary data). These results showed that treatment with 11β-HSD1 inhibitor **10c** improved blood glucose control, indicating that compound **10c** exhibited good in vivo efficacy against type 2 diabetes in a mouse model.

Though **10c** showed favorable profiles in an in vivo mouse model, two key issues proved difficult to address in this series. First, **10c** exhibited poor PK profiles. The highly lipophilic nature of alkyl groups likely contributes to the generally poor metabolic stability of these compounds. Another major concern is the marginal selectivity against the human 11β-HSD2 enzyme. As seen in Table 4, compound **10c** exhibited about 40-fold selectivity for human 11β-HSD1 versus human 11β-HSD2 (IC_{50} = 860 nM) which needs further improvement.

In conclusion, we have identified a series of amide and urea derivatives as novel and potent 11β-HSD1 inhibitors. Several compounds exhibited high in vitro activity toward human 11β-HSD1. Compound **10c** was selected for an in vivo experiment. It exhibited good in vivo efficacy in diabetic ob/ob mice and reduced fasting and non-fasting blood glucose levels after ip dosing. **10c** was

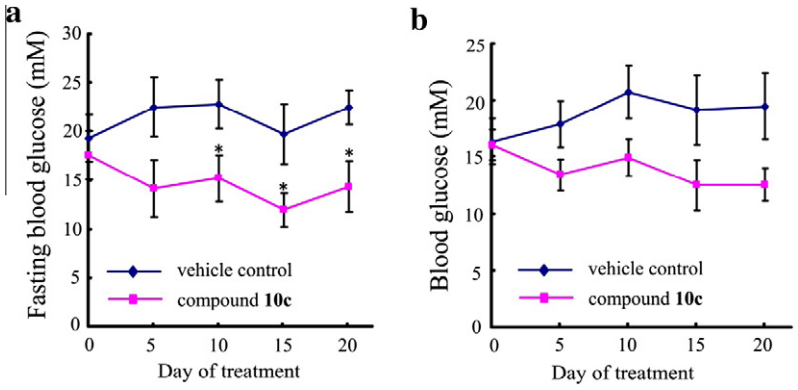


Figure 3. Antidiabetic efficacy of compound **10c** in ob/ob mice. Ob/ob mice were administered with compound **10c** (50 mg/kg) once daily by intraperitoneal injection for 20 days. Fasting (a) and non-fasting (b) blood glucose levels were measured every five days. Values are expressed as means ± SEM for $n = 7$ mice. *P <0.05 versus vehicle control mice.

identified as a highly advanced lead and further optimization of its PK profile and selectivity toward 11β-HSD2 is currently under progress.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.095.

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