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4-(Phenylsulfonamidomethyl)benzamides as potent and selective inhibitors of the 11β-hydroxysteroid dehydrogenase type 1 with efficacy in diabetic ob/ob mice

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ABSTRACT

Selective inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) have considerable potential as treatments for type 2 diabetes. Presented herein are the syntheses, structure–activity relationships, and efficacy evaluation of 4-(phenylsulfonamidomethyl)benzamides as 11β-HSD1 inhibitors. Through modification of our initial lead **5**, we have identified potent and selective 11β-HSD1 inhibitors, such as **11n**, which demonstrated improved glycemic control, decreased serum lipids, and enhanced insulin sensitivity when dosed ip in diabetic ob/ob mice.

Type 2 diabetes (T2D) is a combination of metabolic abnormalities, including hyperglycemia, hyperlipidemia, and insulin resistance. The prevalence of diabetes around the world is estimated to be 2.8% in 2000 and expected to increase at an alarming rate.[1] At present, there are several drugs on the market that partially lower glucose levels and improve insulin sensitivity, however, more effective therapies with fewer side effects are needed.[2] In this respect, numerous research labs are continuing to develop new agents aimed at novel anti-diabetic targets. Over the past few years, one biological target that has attracted significant attention is 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1).

11β-HSD1 is a key enzyme that acts as an NADPH-dependent reductase and converts inactive cortisone into the receptor-active glucorticoid cortisol in humans. It is highly expressed in liver and adipose tissues. Conversely, 11β-hydroxysteroid dehydrogense-2 (11β-HSD2) oxidizes cortisol to cortisone utilizing NAD as a cofactor and is primarily expressed in kidney, colon, and other tissues.[3,4] Inhibition of 11β-HSD2 might lead to sodium retention, hypokalemia, and hypertension,[3] indicating that inhibitors for 11β-HSD1 must be selective over 11β-HSD2.

The connection between 11β-HSD1 and T2D has been demonstrated in mouse genetic models. Mice overexpressing 11β-HSD1 in adipose showed metabolic syndrome-like phenotypes such as central obesity, glucose intolerance, and insulin resistance.[5,6] In contrast, 11β-HSD1 deficient mice were resistant to development of high-fat diet-induced obesity and exhibited improved insulin sensitivity and lipid profiles.[7,8] These data suggest that 11β-HSD1 could be a drug target for the treatment of metabolic syndrome as well as type 2 diabetes.

In the past few years, a number of small molecule inhibitors of 11β-HSD1 have been disclosed, and Incyte's small molecule inhibitor INCB-13739 is currently in phase II clinical trials.[9]

Early in our 11β-HSD1 program, 3-chloro-*N*-*p*-benzenesulfonamide (**5**, Fig. 1) was identified through structure-based virtual screening on the chemical database of our group.[10] The compound is a modest inhibitor of 11β-HSD1, with an IC_{50} of 576 nM in mouse 11β-HSD1 and 1672 nM in human 11β-HSD1. This paper describes the structure–activity relationships (SAR) of a series of analogues to **5** that led to the identification of a potent inhibitor of 11β-HSD1 with in vivo activity.

4-(Phenylsulfonamidomethyl)benzamides were prepared via the route shown in Scheme 1. Protection of 4-(aminomethyl)-benzoic acid **6** as its methyl ester by the usual way of refluxing with acidic methanol gave **7**. Sulfonylation of the amino ester **7** using various arylsulfonyl chlorides in the presence of triethylamine afforded intermediate **9** in excellent yield. Hydrolysis of the methyl esters **9** in methanol using potassium hydroxide gave the carboxylic acids in high yields. Final products **5** and **11** were obtained by treatment of the intermediate acids **10** with various amines using 1-hydroxybenzotriazole and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride as amide coupling reagents, or by converting the carboxylic acids **10** to the corresponding acyl

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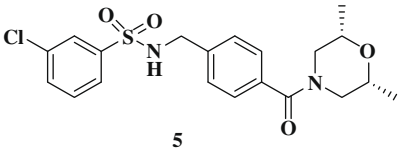


Figure 1. Representative initial compound **5**.

chloride using thionyl chloride followed by reaction with 1-adamantanamine in the presence of pyridine. The noncommercially available N-substituted cycloheptanamines **14** were prepared according to methodologies described in the literature.[11]

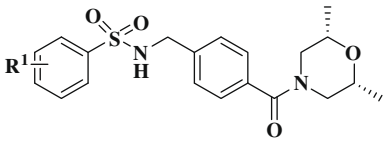
The inhibitory properties of synthesized molecules and the reference compounds (Glycyrrhizic acid and Carbenoxolone) were evaluated in a scintillation proximity assay (SPA)[10,12] (for details, see Supplementary data) with human and mouse 11β-HSD1 (from HEK293 cells transfected with full-length pcDNA3-derived expression plasmid), and selected compounds were evaluated in human and mouse 11β-HSD2 assays.

We initially focused our efforts on modification of the sulfonamide aryl ring (Table 1). Replacement of the 3-Cl with 4-Cl (**11a**) resulted in about twofold loss of potency in both human and mouse enzymatic assays as compared to **5**. However, introduction of 2-Cl (**11b**) or 3-CF_3 (**11c**) only resulted in reduction of potency in mouse assays. Such discrepancies in 11β-HSD1 activity between species might be anticipated, because the mouse and human 11β-HSD1 enzymes display only 79% amino acid identity.[13] Introduction of an additional fluorine atom at the 4-position (**11d**) led to approximately a twofold decrease in potency.

We then turned our attention to modification and replacement of the 2,6-dimethylmorpholine ring (Table 2). Removal of the 2,6-dimethyl group (**11e**) or replacement of the 2,6-dimethylmorpholine with *N*-methylpiperazine (**11f**) resulted in a substantial reduction in activity. When the 2,6-dimethylmorpholine was replaced by piperidine (**11g**), the potency had approximately a threefold increase, while replacement by pyrrolidine (**11h**) or diethylamine (**11i**) had similar potency to **5**.

On the basis of the compound **11g**, we synthesized a cyclohexylamine derivate (**11j**), which drew out the nitrogen atom from

Table 1
Inhibition of 11β-HSD1: sulfonamide aryl modification

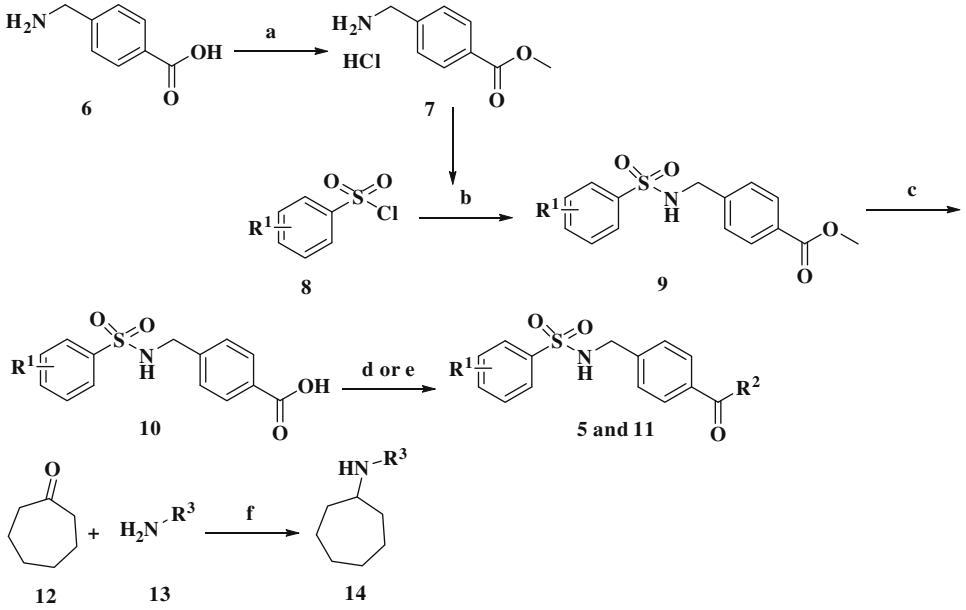


Compound	R^1	IC_{50} (nM) of 11β-HSD1	
		Human	Mouse
5	3-Cl	1672	576
11a	4-Cl	2698	1404
11b	2-Cl	1130	5843
11c	3-CF_3	1208	5614
11d	3-Cl-4-F	2631	1024
Glycyrrhizic acid		10	8

piperidine ring. At the same time, we also synthesized cyclopentylamine and cycloheptylamine derivates (**11k** and **11l**). Compound **11j** had similar potency to **11g**, while decrease of the ring size (**11k**) resulted in a substantial reduction in activity. However, increase of the ring size (**11l**) resulted in about 10-fold improvement of the potency. The most potent compound from this series, the cycloheptylamine analogue **11l**, was a selective inhibitor for 11β-HSD1 and exhibited no appreciable activity against 11β-HSD2 (Table 3).

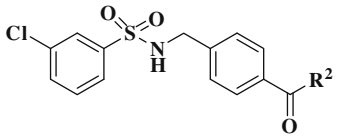
Based on the structure of **11l**, we prepared analogues with alkyl substitute at the nitrogen of the cycloheptylamine (**11m–o**) and replacement of the cycloheptylamine by 1-adamantanamine (**11p**) (Table 3). All of the compounds showed improved potency over **11l**, except **11m** in human assays. The most potent compound, **11n** (human-1 IC_{50} = 2 nM, mouse-1 IC_{50} = 2 nM), exhibited high selectivity versus 11β-HSD2, and was selected for in vivo efficacy evaluation in type 2 diabetic animal models, ob/ob mice.

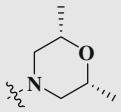
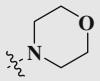
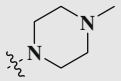
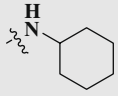
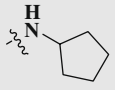
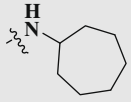
As shown in Figure 2, ob/ob mice displayed significant higher blood glucose, plasma HbA_{1c}, serum insulin, triglyceride and total cholesterol level when compared with its lean littermates (C57 mice). After 4 days of treatment with compound **11n** (50 mg/kg)



Scheme 1. Reagents and conditions: (a) $SOCl_2$, MeOH, reflux; (b) Et_3N, CH_2Cl_2; (c) 1 M NaOH, MeOH, reflux; (d) HOBt, EDCI, CH_2Cl_2; (e) (i) $SOCl_2$, CH_2Cl_2, reflux; (ii) C_5H_5N, 1-adamantanamine; (f) (i) $Ti(O^iPr)_4$; (ii) $NaBH_3CN$.

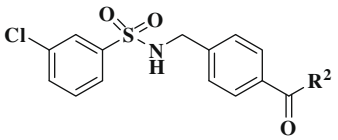
Table 2
Inhibition of 11β-HSD1: 2,6-dimethylmorpholine replacements

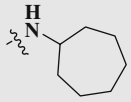
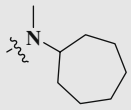
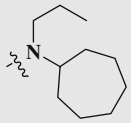
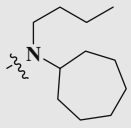


Compound	R^2	IC_{50} (nM) of 11β-HSD1	
		Human	Mouse
5		1672	576
11e		14,490	3379
11f		15,900	1319
11g		647	131
11h		1733	480
11i		2106	395
11j		411	302
11k		8512	732
11l		49	15

by intraperitoneal injection, the fasting blood glucose level of ob/ob mice were reduced by 42%, compared with vehicle control mice ($P < 0.01$). A significant reduction of 36% in non-fasting blood glucose level was also observed in ob/ob mice after 8 days treatment with compound **11n**. During the 23 days treatment, compound **11n** reduced the fasting and non-fasting blood glucose level in ob/ob mice by an average rate of 35% and 28%, respectively (Fig. 2a and b). Consistent with the reduced fasting and non-fasting blood glucose levels, chronic treatment with 50 mg/kg compound

Table 3
Inhibition of 11β-HSD1 and 11β-HSD2 of compounds **11l–11p**



Compound	R^2	IC_{50} (nM) of 11β-HSD1		IC_{50} (nM) of 11β-HSD2	
		Human	Mouse	Human	Mouse
11l		49	15	>10,000	>10,000
11m		638	2	2487	>1,000,000
11n		2	2	>1,000,000	>1,000,000
11o		47	2	>100,000	>1,000,000
11p		14	10	>100,000	>1,000,000
Glycyrrhizic acid		10	8	1	
Carbenoxolone					82

11n for 23 days decreased the Hemoglobin A1c (HbA_{1c}) level by 0.57% ($P < 0.05$), compared with vehicle mice, which reflected the beneficial effect of compound **11n** on glycemic control in ob/ob mice (Fig. 2c). Treatment of ob/ob mice with compound **11n** for 23 days resulted in a significant reduction of 28% in serum insulin level compared with vehicle mice, which suggested enhanced insulin sensitivity (Fig. 2d). Moreover, the serum triglyceride and total cholesterol level were also lowered significantly by the 23 days treatment of compound **11n** in ob/ob mice (Fig. 2e and f). Therefore, chronic treatment with selective 11β-HSD1 inhibitor compound **11n** improved glycemic control, decreased serum lipids and enhanced insulin sensitivity in ob/ob mice, indicating that compound **11n** exhibited good in vivo efficacy against type 2 diabetes in a mouse model.

In summary, we have identified a novel class of 11β-HSD1 inhibitors containing a 4-(phenylsulfonamidomethyl)benzamide core structure. Our initial lead compound **5**, in this series, was modestly potent. Significant improvements in potent were achieved by modification of the parent compound **5**. Some compounds showed high potency in 11β-HSD1 and exhibited high selectivity for 11β-HSD1 versus 11β-HSD2. The most potent compound **11n** was selected for an in vivo experiment, and it demonstrated improved glycemic control, decreased serum lipids, and enhanced insulin sensitivity in diabetic ob/ob mice.

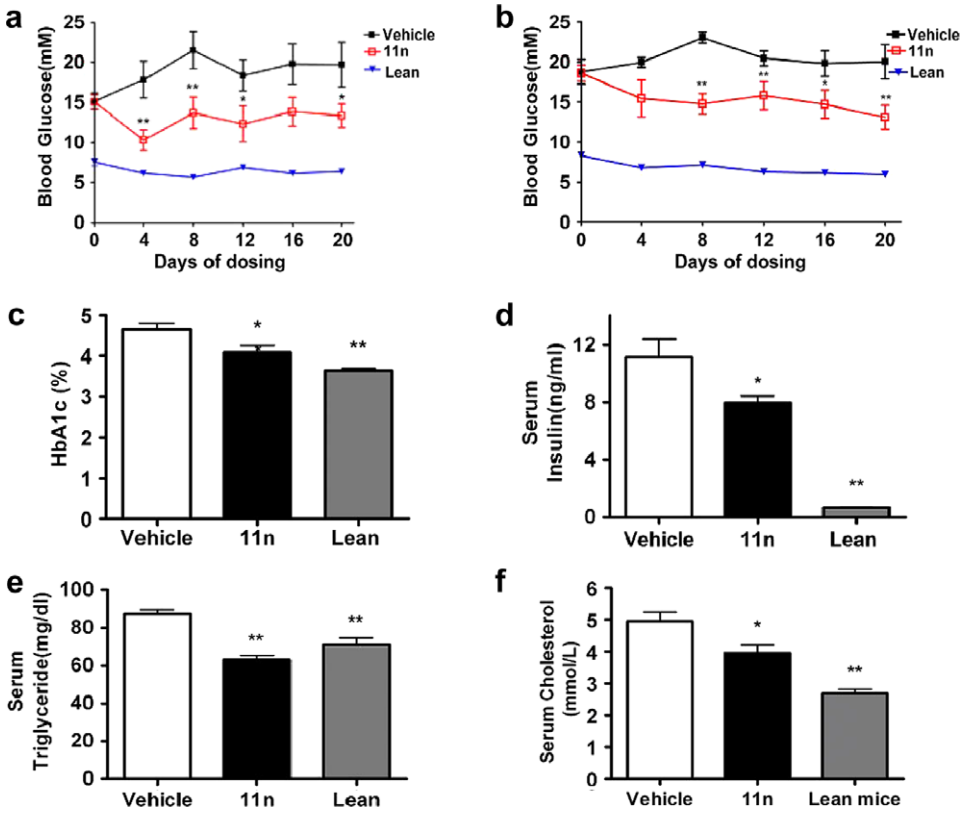


Figure 2. Anti-diabetic efficacy study of compound **11n** in ob/ob mice. Ob/ob mice were administered with compound **11n** (50 mg/kg) once daily by intraperitoneal injection for 23 days. Fasting (a) and non-fasting (b) blood glucose levels were measured every four days. HbA_{1c} (c), serum insulin (d), triglyceride (e), and total cholesterol (f) levels were measured after 23 days treatment. Values are expressed as means ± SEM for $n = 8$ mice. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus vehicle control mice.

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.2009.05.033.

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