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3-Amino-*N*-adamantyl-3-methylbutanamide derivatives as 11β-hydroxysteroid dehydrogenase 1 inhibitor

Younho Lee [a], Yong June Shin [b], Soon Kil Ahn [c,*]

[a] College of Pharmacy and Yonsei Institute of Pharmaceutical Sciences, Yonsei University, 162-1 Songdo-dong, Yeonsu-gu, Incheon 406-840, Republic of Korea
[b] R&D Center, Ahn Gook Pharm., Gyeonggi Bio-Center, Suwon, Gyeonggi-do 443-766, Republic of Korea
[c] Institute for New Drug Development, Division of Life Sciences, Incheon National University, Incheon 406-772, Republic of Korea

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ABSTRACT

Many adamantane derivatives have been demonstrated to function as 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitors. 3-Amino-*N*-adamantyl-3-methylbutanamide derivatives were optimized by structure-based drug design. Compound **8j** exhibited a good in vitro and ex vivo inhibitory activity against both human and mouse 11β-HSD1.

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The symptoms of Cushing's syndrome include metabolic complications such as insulin resistance, obesity, dyslipidemia, hypertension, and type 2 diabetes. This disease is caused by excessive levels of the active glucocorticoid hormone, cortisol.[1] In tissues, the cortisol level is regulated by two 11β-hydroxysteroid dehydrogenase (11β-HSD) isozymes. The type 1 enzyme (11β-HSD1) is an NADPH-dependent reductase that converts the inactive form glucocorticoid, cortisone into the active form, cortisol in humans. This enzyme is highly expressed in liver and adipose tissues. In contrast, 11β-HSD type 2 (11β-HSD2) performs oxidative functions and expressed in the kidneys, colon, and sweat and salivary glands. The excess cortisol hormone induces metabolic problem such as increased lipid mass and insulin resistance by binding to the glucocorticoid receptor (GR) (Fig. 1).

11β-HSD1 alters its structure from a dimer to that of a tetramer for substrate binding. The cofactor, NADPH and substrate, cortisone are used to produce cortisol. The catalytic triad of Ser170, Tyr183, and Lys187 catalyzes the conversion of cortisone to cortisol. Other amino acids residues are important for the substrate change and inhibitors binding as well.[2,3] Many types of inhibitors that have been developed in clinical phases have demonstrated improvements in type 2 diabetes, obesity, and blood lipid profiles.[4–9]

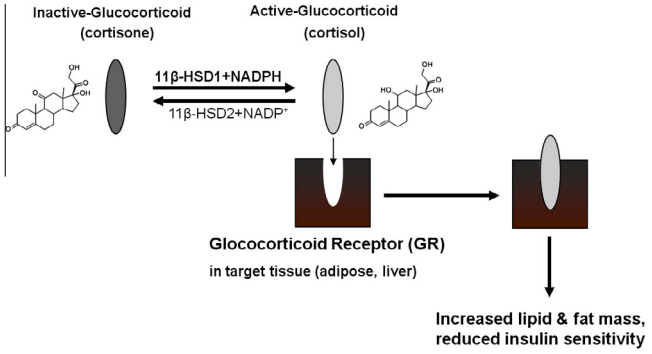

Figure 1. The function of 11β-HSD and glucocorticoid.

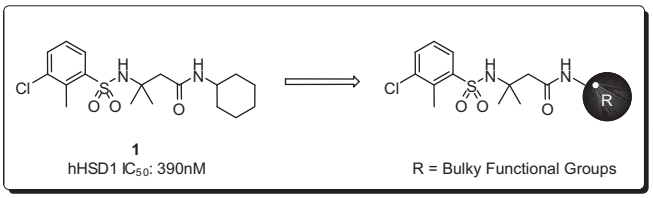

Figure 2. Initial hit compound **1** and modification.

* Corresponding author. Tel.: +82 32 835 8248; fax: +82 32 835 0763.
E-mail address: skahn@incheon.ac.kr (S.K. Ahn).

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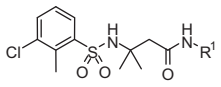
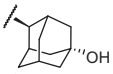
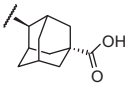
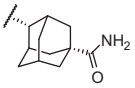
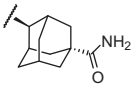
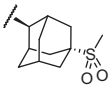
Table 1
In vitro human and mouse 11β-HSD1 inhibitory activity of methylbutanamide derivatives with an adamantyl group

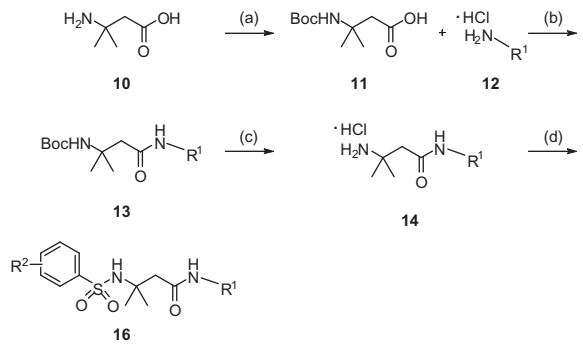


Compound	R^1	11β-HSD1 IC_{50} (nM)	
		Human	Mouse
1		390	N/D[a]
2		190	N/D
3		80	640
4		4	113
5		4	70
6		57	>1000
7		>1000	N/D
8		1	12
9		54	>1000
AMG221	—	7	470

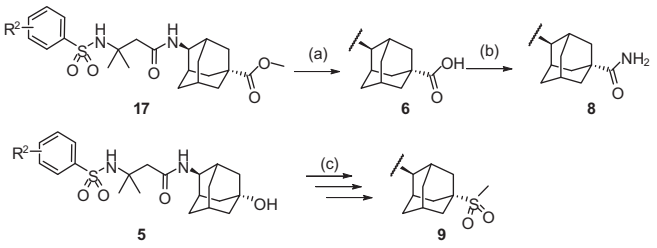
[a] N/D; not determined.

Recently, positive result from a 28-day phase IIa clinical trial with a potent 11β-HSD1 inhibitor, INCB013739 were reported by Incyte.[10] However, many 11β-HSD1 inhibitors under development including INCB013739 only exhibit inhibitory activity against human 11β-HSD1 with no inhibition against the mouse enzyme. This lack of cross-species activity causes great difficulties in pre-clinical evaluation for the development of novel drug candidates.

An ongoing efforts to discover novel inhibitors active against both human and mouse 11β-HSD1 involves structure based drug design and the evaluation of their in vitro and ex vivo activity. In this Letter, we report the discovery of 3-amino-*N*-adamantyl-3-methylbutanamide derivatives as a potent 11β-HSD1 inhibitor.



Scheme 1. Reagents and conditions: (a) Boc_2O, 0.5 N NaOH, 1,4-dioxane, rt, 24 h; (b) BOP-Cl, TEA, DCM, rt, 12 h; (c) 4 M HCl in dioxane, ethyl acetate, rt, 2 h; (d) TEA, DCM, benzene sulfonyl chloride derivatives, rt. R^1 of **12**: Cyclohexane, cycloheptae, 1-adamantane, 2-adamantane, 5-carboxylic ester 2-adamantamine or 5-hydroxy-2-adamantamine. R^2 of **16**: Diverse *ortho*, *meta*, or *para* substituted benzenes.



Scheme 2. (a) 2N-NaOH, THF/EtOH (1:1), rt, overnight; (b) aq NH_4OH, HOBt, EDCI, DCM, rt, 20 h; (c) (i) methanesulfonyl chloride, TEA, DCM, rt, 12 h; (ii) $NaSCH_3$, MeOH, 80 °C, 12 h; (iii) aq $NaBO_34H_2O$, AcOH, rt, 1 h. R^2 of **5** and **17**: Diverse *ortho*, *meta*, or *para* substituted benzenes.

An initial hit compound **1** from an in house chemical library was identified with a moderate potency in human liver microsome 11β-HSD1 (IC_{50}: 390 nM). The activity was improved by changing the cyclohexane functional group (Fig. 2). The inclusion of more bulky groups such as cycloheptane and adamantane improved the 11β-HSD1 inhibitory activity by 2- and 5-folds, respectively (**2** and **3**). Next, we explored substituted adamantine derivatives. 2-Adamantane (**4**) exhibited a potent 11β-HSD1 inhibitory activity in human liver microsomes. However, the compound exhibited insufficient activity in mouse liver microsomes. Therefore, efforts were focused on substitution at the 5-position of adamantane to improve the activity in mice (Table 1). That positional change strategy was also applied for the other adamantane derivatives.[5,8,9,11–13]

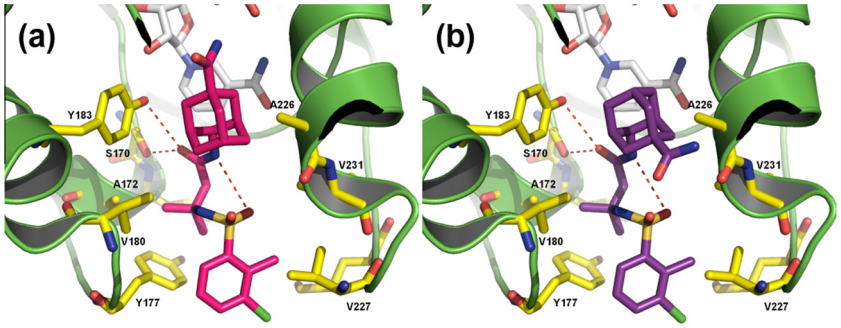


Figure 3. The docking models of compound **7** and **8**. (a) Compound **8** is depicted in magenta with yellow binding site residues. The dash line indicates the hydrogen bonding interactions. (b) Compound **7** is presented in purple color. The terminal amide group exhibits steric hindrance against the A226.

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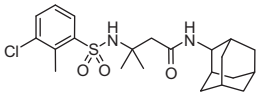
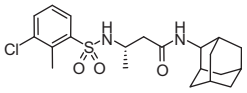
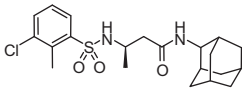
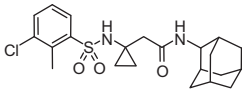
Table 2
In vitro human and mouse 11β-HSD1 inhibitory activity of methylbutanamide analogs with an adamantyl group

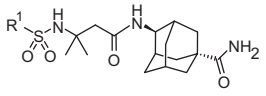
Compound	Structure	11β-HSD1 IC_{50} (nM)	
		Human	Mouse
4		4	113
4a		89	N/D[a]
4b		53	N/D
4c		12	94
AMG221	—	7	470

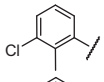
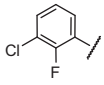
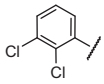
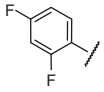
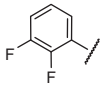
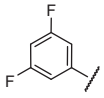
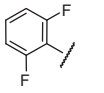
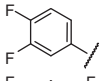
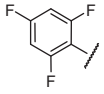
[a] N/D; not determined.

We attempted to optimize the activity by attaching hydrophilic functional groups to adamantane. Compound **5** exhibited potent human 11β-HSD1 inhibitory activity and improved mouse activity. In particular, the adamantane amide (**8**) demonstrated improved inhibitory activity against the 11β-HSD1 of both species. However, adamantane carboxylic acid (**6**) and methyl sulfone (**9**) decreased the 11β-HSD1 inhibitory activity in both species.[14,15]

Figure 3 represents the binding model of the most active compound **8**. As previously mentioned, the amino acids, S170 and Y183, are important for inhibitor binding. Two hydrogen bonding interactions exist between the carbonyl oxygen of compound **8** and the hydroxyl groups of serine and tyrosine. There are two van der Waals interactions. One occurs between the adamantane group and Y183 and A226 residues. The dimethyl group and A172 and Y177 residues also exhibit a similar interaction. In addition, phenyl group and Y-177 residue provide π–π interaction. As shown in our binding model, the intra-molecular hydrogen bonding between the oxygen of the sulfone group and the hydrogen of the amide group maintains the compound in V-shape conformation. The conformation fixed by hydrogen bonding helps the flexible compound bind properly to the active site pocket (Fig. 3a).[16]

Table 3
In vitro human and mouse 11β-HSD1 inhibitory activity of sulfonyl methylbutanamide derivatives with adamantyl groups



Compound	R^1	11β-HSD1 IC_{50} (nM)		Metabolic Stability (Remain% @ 30 min)	
		Human	Mouse		
8		1	12	77	99
8a		1	10	91	40
8b		1	9	76	5
8c		1	22	96	25
8d		1	9	91	8
8e		7	99	100	14
8f		2	16	86	24
8g		2	196	96	31
8h		1	28	94	14

(*continued on next page*)

Table 3 (*continued*)

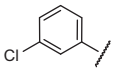
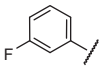
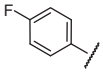
Compound	R^1	11β-HSD1 IC_{50} (nM)		Metabolic Stability (Remain% @ 30 min)	
		Human	Mouse		
8i		1	46	99	57
8j		2	21	93	99
8k		2	28	97	13
8l		1	24	94	26
AMG221	—	7	470	—	—

Table 4
Selectivity and toxicity test results of **8j** including the mouse ex vivo results

	11β-HSD2 (μM)	MTT (293) (μM)	CYP450[a] (μM)	hERG (μM)	Ex vivo	
					Liver (%)	Fat (%)
8j	>10	>50	>10	>100	68	11

[a] CYP450 1A2, 2D6, 2C9, 2C19, and 3A4 were tested.

The 5-amide-2-adamantamine has two diastereomers, the *Z* and *E* forms (**7** and **8**). The *E* form is more active than the *Z* form. Whereas the *E* form occupies the 11β-HSD1 pocket properly, the 5-substituted amide group of the *Z* form exhibits a steric hindrance against the A226 residue of the binding site (Fig. 3b).

Compound **1** and its derivatives were synthesized as shown in Scheme 1.[17] A Boc-protected amine (**11**) obtained from commercially available 3-amino-3-methylbutanoic acid (**10**) was coupled with diverse amines such as cyclic amine, adamantamine, 5-hydroxy-2-adamantamine or 5-carboxylic ester 2-adamantamine. The compound **4** and derivatives (**4a–c)** were made by coupling with substituted benzene sulfonylchloride after Boc-deprotection (Scheme 1). Compound **8** and **9** were synthesized from the 5-carboxylic ester 2-adamantamine derivative (**17)** or 5-hydroxy-2-adamantamine derivative (**5**), respectively (Scheme 2).[18,19]

Next we evaluated the effect of the dimethyl group of compound **4** on compound's inhibitory effects against 11β-HSD1. Compounds that had the (*S*) and (*R*) form of the methyl group (**4a** and **4b**) demonstrated approximately 10–20 folds decreased activities. However, a compound containing a cyclopropyl group **4c** exhibited only slightly reduced effects in human microsomes and similar activity in those of mice. From these results, we can see fully occupied groups within the hydrophobic pocket (around A172, Y177) are preferred for the inhibitor binding. The most active compound **4** in humans, was used for further optimization (Table 2).

Finally we sought to improve the 11β-HSD1 inhibitory activity by adopting several substituted benzene derivatives (**8a–8l**). All analogs demonstrated one digit nano-molar IC_{50} values against human 11β-HSD1. However, the mouse 11β-HSD1 inhibitory activities did not correlate with the compound structure. We doubted the lack of correlation and reached a tentative conclusion. Our assay was conducted using liver microsomes as a source of 11β-HSD1. However, liver microsomes contain many other enzymes such as CYP450 responsible for the degradation of xenobiotics, which affect the stability of compounds and the inhibitory activity of tested compounds (Table 3).[20]

The 11β-HSD2 assay and some toxicity related assays were carried out for the potent and metabolically stable compound **8j**. The compound did not inhibit 11β-HSD type 2 and had no toxic effects in in vitro test. The ex vivo effect on mouse tissue was approximately 68% inhibition in mouse liver tissue at a single gavage dose of 45 mg/kg, while weak in fat tissue (Table 4).[21]

In conclusion, the 3-amino-*N*-adamantyl-3-methylbutanamide derivatives exhibit good inhibitory effects against both human and mouse 11β-HSD1 and no toxic effects. Preclinical development is underway for a number of the derivatives.

Acknowledgments

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14. The in vitro activity of 11β-HSD1 derived from microsomal fractions was measured using the HTRF assay (Cisbio, Marcoule, France) according to the manufacturer's instructions. Briefly, different concentrations of compounds were added to 96-well plates, followed by the addition of TE buffer (20 mM Tris buffer and 5 mM ethylenediaminetetraacetic acid, pH 6.0) containing 200 μM NADPH and 160 nM cortisone (Sigma, St. Louis, MO). The reactions were initiated by the addition of human (20 μg), mouse (20 μg), or monkey (320 μg) microsomal fractions, and were allowed to incubate for 2 h at 37 °C. Europium (Eu^{3+}) cryptate and XL665-conjugated cortisol were then added to each well and incubated for an additional 2 h at room temperature. The cortisol concentration was calculated using a calibration curve; data were obtained from at least 2 determinations. The IC_{50} values were calculated from dose response curves using GraphPad Prism software.
    To measure 11β-HSD2 activity, human kidney microsomes were incubated in 96-well plates in the presence of cortisol (Sigma, St. Louis, MO) with or without compounds. Enzyme activity was determined by measuring the amount of cortisone product using LC–MS.
    A luminescent CYP inhibition assay was performed using P450-Glo assay system according to the manufacturer's instructions (Promega, Madison, WI, USA).
    Human and mouse liver microsome stabilities were determined as follows. The compounds were incubated with liver microsomes. At 30 min time points, aliquots were quenched and analyzed by LC–MS.
15. All data are presented as mean ± SEM. Differences between groups were determined by one-way ANOVA followed by Duncan's multiple comparison test. Data were considered to be statistically significant at $p < 0.05$.
16. The binding models of the 11β-HSD1/compound **7** and **8** complexes were described in Figure 3. In docking studies, the structure of 11β-HSD1 was taken from PDB 2ILT (http://www.rcsb.org), in which the structure was solved in a complex bound to adamantane sulfone inhibitor. Based on the adamantane amide coordinates, the compound structure was superimposed to an adamantane amide inhibitor in the X-ray crystal complex. The initial complex was optimized with 1000 steps of steepest decent and 3000 steps of conjugate gradient while holding the 11β-HSD1 heavy atoms restrained to their initial positions by means of a harmonic force constant of 1 kcal mol^{-1} $Å^{-2}$ using CHARMm in Accelrys Discovery Studio 2.5.

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20. *All novel synthetic compounds gave satisfactory analytical and spectral data. Selected data for* ***8j***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88–7.92 (m, 1H), 7.54–7.60 (m, 1H), 7.23–7.29 (m, 1H), 7.18–7.22 (m, 1H), 6.36 (d, J = 8.0 Hz, 1H), 5.82 (s, 1H), 5.60 (br s, 1H), 5.30 (br s, 1H), 4.08 (t, J = 3.8 Hz, 1H), 2.49 (s, 2H), 2.11 (s, 2H), 1.96–2.05 (m, 5H), 1.87–1.91 (m, 4H), 1.63 (d, J = 13.2 Hz, 2H), 1.25 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.2, 170.1, 159.5, 157.8, 135.5, 131.6, 129.6, 125.3, 117.6, 55.8, 55.4, 52.9, 47.8, 39.2, 39.0, 31.7, 30.5, 27.4, 27.2; Anal. Calcd for $C_{22}H_{30}FN_3O_4S$: C, 58.52; H, 6.70; N, 9.31. Found: C, 58.63; H, 6.72, N, 9.28.
21. For ex vivo 11β-HSD1 activity analysis, male C57BL/6J mice were randomly assigned to 2 groups based on body weight. Compound **8j** was suspended in 0.5% carboxymethylcellulose (CMC) and given as a single gavage dose of 45 mg kg^{-1}; control animals were given vehicle (0.5% CMC) only. The animals were sacrificed 2 h after dosing, and the liver and epididymal fat tissues were isolated immediately, frozen in liquid nitrogen, and stored at −80 °C. At the time of the assay, frozen tissues were partially thawed and dissected into 30–40 mg samples and placed directly into 24-well plates containing pre-warmed DMEM supplemented with 100 μM NADPH and 1 μM cortisone. After 3-h incubation at 37 °C, the cortisol concentration in the media was measured using the HTRF cortisol assay; inhibition of 11β-HSD1 activity in tissues was determined relative to that in vehicle-treated mice.