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Discovery and biological evaluation of adamantyl amide 11β-HSD1 inhibitors

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Abstract—A series of adamantyl amide 11β-HSD1 inhibitors has been discovered and chemically modified. Selected compounds are selective for 11β-HSD1 over 11β-HSD2 and possess excellent cellular potency in human and murine 11β-HSD1 assays. Good pharmacodynamic characteristics are observed in ex vivo assays.

Glucocorticoids (e.g., cortisol, corticosterone) are hormones that regulate pathways involved in stress and metabolic signalling. The effects of glucocorticoid excess are evident in Cushing's syndrome, which causes metabolic and cardiovascular abnormalities such as central obesity, insulin resistance, dyslipidaemia and hypertension and CNS manifestations such as depression and cognitive impairment.[1] At the tissue level, glucocorticoid activity is controlled by the 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes, which 'gate' access of these steroids to their nuclear receptors.[2] 11β-HSD1 is present predominantly in the liver, adipose tissue and brain and converts inactive cortisone to cortisol; 11β-HSD2 is expressed mainly in the kidney and catalyzes the reverse reaction. 11β-HSD1 knockout mice display enhanced hepatic insulin sensitivity and reduced gluconeogenesis and glycogenolysis, suggesting that inhibition of 11β-HSD1 will be a useful treatment in type 2 diabetes.[3] Furthermore, these mice have low serum triglycerides and increased HDL cholesterol and apo-lipoprotein A1 levels, suggesting that inhibitors may be useful in the prevention and treatment of atherosclerosis.[4] 11β-HSD1 knockout mice are also protected against age-related cognitive impairment, suggesting that inhibitors may be of therapeutic use in the treatment of diseases such as Alzheimer's disease, which are characterised by cognitive dysfunction.[5]

In recent years considerable activity in the pharmaceutical industry has led to the discovery of several classes of 11β-HSD1 inhibitors. Early examples are non-selective steroidal molecules such as carbenoxolone (**1**), which is also a potent inhibitor of 11β-HSD2.[6] Numerous examples of non-steroidal inhibitors have also been disclosed.[7] These include triazoles such as compound 544 (**2**), sulfonamides like BVT-14225 (**4**) and adamantyl carboxamides such as **3** (Fig. 1).[8] Compound 544 has been shown to produce beneficial effects in animal models of atherosclerosis and of type 2 diabetes, with lowering of plasma triglycerides, glucose, rate of body weight gain and food intake.[9]

We have discovered and modified a series of adamantyl amides that display inhibition of 11β-HSD1 in vitro and in vivo. Compounds were tested for inhibition in mammalian cells stably transfected with human 11β-HSD1 or human 11β-HSD2 using a scintillation proximity assay (SPA).[10] Selected compounds were also tested in cells expressing murine 11β-HSD1.

A primary in vitro compound screen was performed on a selection of putative 11β-HSD1 inhibitors identified

Keywords: Metabolic syndrome; 11β-HSD1; 11β-HSD2; Inhibitor; Adamantane; Amide; Diabetes; 11β-Hydroxysteroid dehydrogenase; Enzyme inhibition; Hsd1.
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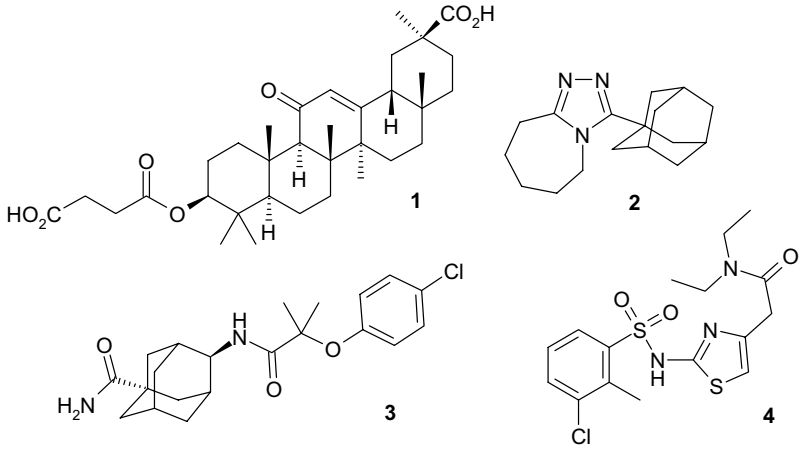
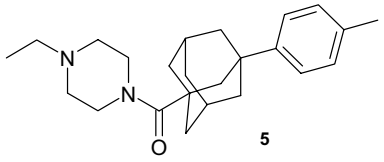


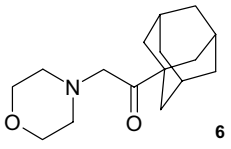
Figure 1. Selected 11β-HSD1 inhibitors.

from a field-based in silico screen.[11] From a subsequent focused screen of selected cyclic amines two closely related compounds containing a carbonyl group adjacent to an adamantane were identified as 11β-HSD1 inhibitors. Both compounds display sub-micromolar potency in cellular assays and are selective for 11β-HSD1 (Fig. 2).

The adamantyl moiety appears to be a privileged structure present within many 11β-HSD1 inhibitors disclosed in the patent and scientific literature.[12] However, the moiety is known to be metabolically labile due to hydroxylation at the 3- and 5-positions.[13] Initial medicinal chemistry focused on the synthesis of modified adamantanes (Table 1a). Hydroxylated analogues were prepared to evaluate the potency of the likely primary metabolites formed after first pass metabolism in the liver. Analogues were prepared according to Scheme 1. For compounds **12**, **13** and **16**, ethyl piperazine was replaced by piperidine. The initial hit (**5**) contains a 4-tolyl group at the 5-position of adamantane and it is more potent than unsubstituted adamantane in the ethyl piperazine series (**10**). Replacement of methyl with a methoxy group at the para position of the phenyl group (**14**) results in a slight loss of potency and selectivity compared to the screening hit (**5**). Introduction of a methylene unit between the adamantyl and phenyl groups (**11**) also leads to a significant decrease in potency, suggesting that the distance between these two groups is key to retaining potency. Attachment to the 1- and 2-positions of adamantane was explored and the resulting piperidine compounds were equipotent



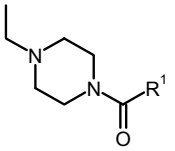
hHSD1 IC_{50} = 82nM
hHSD2 %inh @ 10μM = 0%

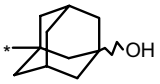
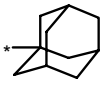
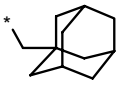
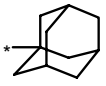
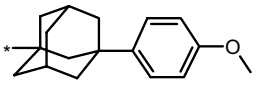
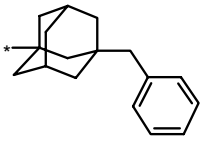
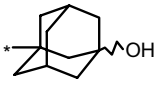


hHSD1 IC_{50} = 660nM

Figure 2. Hits from focused screen.

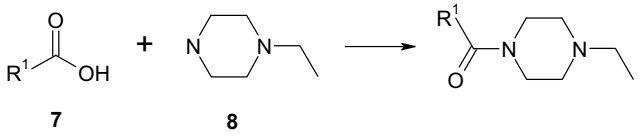
Table 1a. Human 11β-HSD1 and 11β-HSD2 inhibition for compounds **9**–**16**



Compound	R^1	hHSD1 IC_{50} (μM)	hHSD2 %inh at 10 μM
9		53	ND
10		0.8	11
11		4.1	1
12[a]		0.2	39
13[a]		0.2	0
14		0.6	51
15		1.0	0
16[a]		7.3	9

[a] Ethyl piperazine replaced by piperidine.

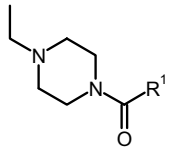
(**12** and **13**) and approximately fourfold more potent than the adamant-1-yl derivative from the ethyl piperazine series (**10**). The corresponding adamant-2-yl

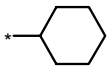
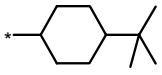
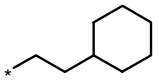
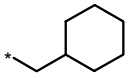
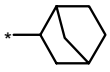
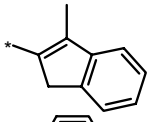
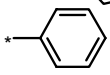
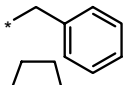
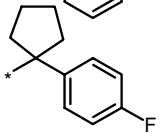


7 **8**

Scheme 1. Reagents and conditions: **7**, DMF, CDI, 60 °C, 1 h, then **8**, 60 °C, 4 h (20–80%).

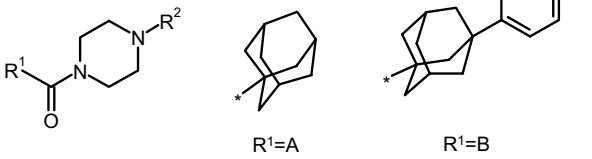
Table 1b. Human 11β-HSD1 and 11β-HSD2 inhibition for compounds **17**–**27**



Compound	R^1	hHSD1 IC_{50} (μM)	hHSD2 %inh at 10 μM
17		>100	0
18		53	0
19		>100	0
20		20	0
21		48	0
22		>100	40
23		44	0
24		15	0
25		>100	0
26		7.2	11
27		12	0

compound in the ethyl piperazine series was not prepared. Hydroxylation of the adamantyl group at the 5-position led to a dramatic reduction in cellular potency in the ethyl piperazine series (**9**), suggesting that this position would have to be blocked by a suitable group to maintain potency in vivo.

Table 2. Human 11β-HSD1 and 11β-HSD2 inhibition of compounds **5**, **10** and **28**–**51**



R^1=A R^1=B

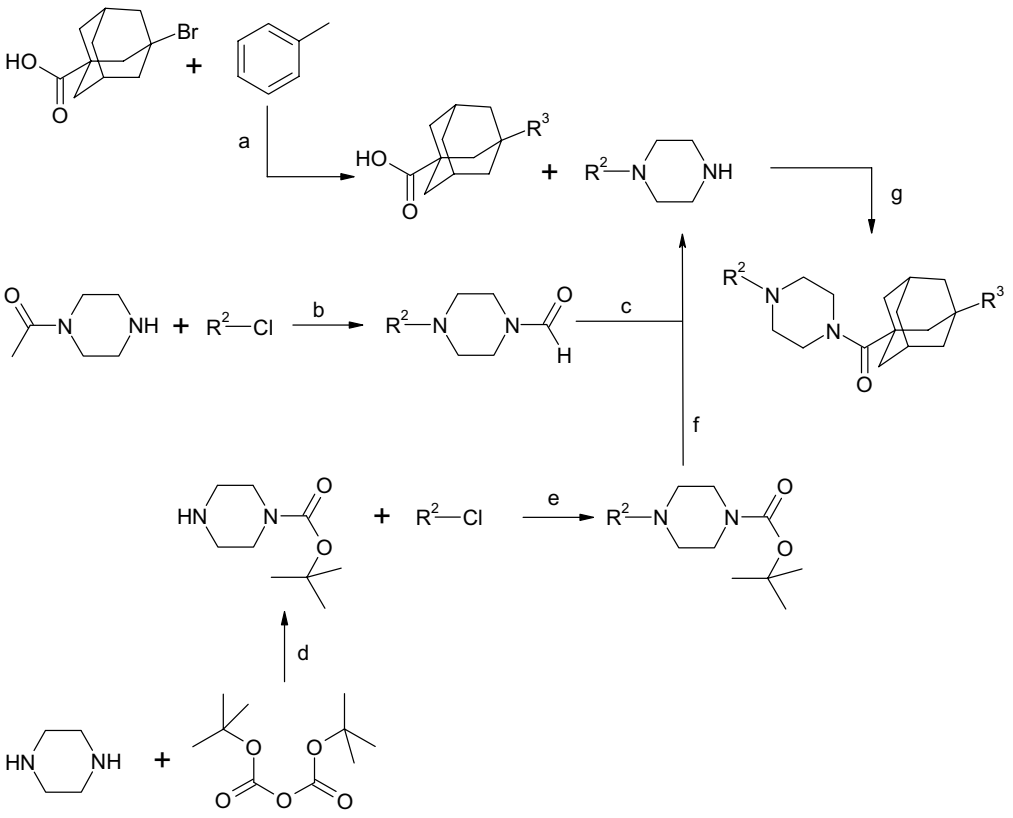
Compound	R^1	R^2	hHSD1 IC_{50} (μM)	hHSD2 %inh at 10 μM
10	A	CH_2CH_3	0.8	0
5	B	CH_2CH_3	0.08	0
28	A	H	8.2	31
29	A	CH_3	0.7	13
30	A	CH_2Ph	7.2	7
31	B	CH_2Ph	1.5	80
32	A	Ph	>100	0
33	B	Ph	7.3	ND
34	A	COPh	>100	20
35	B	COPh	36	0
36	A	CH(CN)Ph	24	39
37	B	CH(CN)Ph	2.1	48
38	A	$CH(CN)CH(CH_3)_2$	20	45
39	B	$CH(CN)CH(CH_3)_2$	10	56
40	A	SO_2Ph	>100	35
41	B	SO_2Ph	64	48
42	A	SO_2CH_2Ph	13	39
43	B	SO_2CH_2Ph	7.7	26
44	A	CH_2pyridin-4-yl	7.2	0
45	B	CH_2pyridin-4-yl	8.1	0
46	A	CH_2CH_2OH	1.5	31
47	B	CH_2CH_2OH	0.5	40
48	A	$CH_2CH_2OCH_3$	12	7
49	B	$CH_2CH_2OCH_3$	2.1	41
50	A	CH_2CH_2Ph	47	0
51	B	CH_2CH_2Ph	1.9	53

ND, not determined.

We explored whether the adamantyl group could be replaced by a selection of saturated and unsaturated groups. A series of acyl piperazine analogues were prepared by coupling substituted carboxylic acids to ethyl piperazine (Scheme 1).

The results suggest that adamantane or a similar saturated fused ring system is a favoured group adjacent to the carbonyl of the piperazine amide (Table 1b). All of the replacements tested resulted in substantial loss of potency and selectivity (e.g., compared with **5** or **10**), suggesting that the adamantyl group occupies a specific lipophilic pocket within the enzyme active site.

Our initial efforts to replace the adamantyl group were unsuccessful. However, since compounds containing an adamantane display good cellular potency, we explored whether it was possible to improve potency by modifying the 4-position of the piperazine in both the adamantyl and *p*-tolyl series (Table 2). Our initial screening hit (**5**) contains an ethyl group at this position suggesting that a similar small alkyl group may be favoured. We explored whether the addition of H-bond

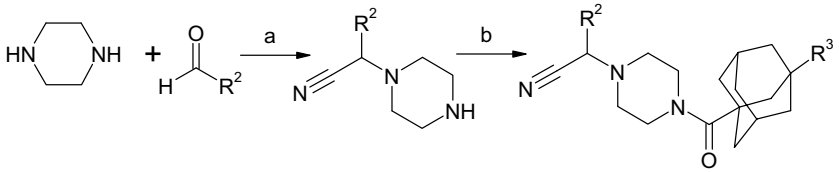


Scheme 2. Reagents and conditions: (a) $ZnCl_2$, reflux, 2 h (80%); (b) $R^2 = PhCH_2CH_2$, 4-py: EtOH, KI, reflux, 50–72 h; (c) HCl, reflux, 2–5 h (50–70%); (d) $R^2 = CH_3OCH_2CH_2$: *i*-PrOH, rt, 30 min, then Et_3N, KOH; (e) EtOH, $NEt(i\text{-}Pr)_2$, Δ, 72 h, then Et_3N, KOH; (f) MeOH, gaseous HCl, rt, then Et_3N, KOH; (g) see Scheme 1.

acceptors and aromatic groups could increase potency. A range of analogues containing alkyl, phenyl and pyridinyl groups fused to linkers were prepared according to Scheme 2. The majority of analogues were prepared directly from commercially available starting materials. Nitrile substituted analogues were prepared by reacting the appropriate aldehyde with piperazine in the presence of KCN (Scheme 3).

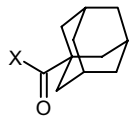
In both the adamantyl and *p*-tolyl series only the addition of small unbranched alkyl groups to the piperazine is tolerated. Replacement of ethyl by methyl in the adamantyl series (**29**) led to a modest increase in potency. However, the addition of phenyl, benzyl or phenylethylene was detrimental to potency. Introduction of H-bond acceptor groups such as carbonyl, sulfonyl or nitrile generally reduced potency, suggesting this region of the parent molecule sits within a tight hydrophobic binding site. Compounds containing H-bond acceptors at the 4-position of the piperazine also displayed increased inhibition of 11β-HSD2. In general, the *p*-tolyl series displayed greater potency than the adamantyl series, suggesting that further modification of the group at the 5-position of the adamantane may yield selective compounds with good potency and enhanced metabolic stability.

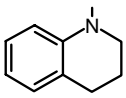
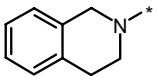
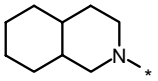
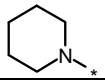
In the adamantyl series, replacement of the ethyl piperazine with piperidine led to a significant increase in potency and retention of selectivity (Table 1a, e.g., **13** vs **10** and **16** vs **9**). Further replacement of the ethyl piperazine was investigated by purchasing commercially available analogues (Table 3). Replacement of ethyl piperazine with pyrrolidine (**52**) marginally increased cellular potency compared with **10**, while replacement with octahydroisoquinoline (**55**) was approximately equipotent. Replacement with dihydroisoquinoline (**54**) significantly reduced potency, further suggesting that only alkyl groups are favoured substituents at the 3- and 4- positions of the piperazine or piperidine rings. These results



Scheme 3. Reagents and conditions: (a) R^2 = i-Pr, Ph: HCl, aq KCN, *i*-PrOH, rt, 1 h, then CCl_4 (70–80%); (b) see Scheme 1.

Table 3. Modifications to piperidine



Compound	Supplier	X	hHSD1 IC_{50} (nM)	hHSD2 %inh at 10 μM
52	Chembridge		498	37
53	Chembridge		1860	0
54	Chembridge		5600	ND
55	Enamine		846	18
13	see Scheme 1		193	0

ND, not determined.

Table 4. Comparison of inhibition between human and murine 11β-HSD1

Compound	hHSD1 IC_{50} (nM)	mHSD1, IC_{50} (nM)
5	82	81
13	193	134
10	756	300
20	20,000	1200
46	1470	494
47	498	43
53	1860	481
55	846	230

suggest that the cyclic amine ring binds to the enzyme in a hydrophobic pocket.

Since in vivo efficacy is typically assessed in murine models of metabolic disease, the potency of selected compounds was determined for murine 11β-HSD1 (Table 4). In general, compounds display greater potency against the murine enzyme. Of particular note are compounds **46** and **47**, which display significantly greater potency for murine than human 11β-HSD1. Both compounds contain a hydroxyethyl substituent at the 4-position of the piperazine, suggesting that the polar hydroxyl group may make a favourable interaction in the murine 11β-HSD1 active site. The active sites of murine and human 11β-HSD1 are reasonably conserved, although key residues involved in substrate binding are significantly different. Tyrosine 177 in human is replaced by glutamine in murine, while alanine 226 is replaced by glutamic acid. It is possible that the hydroxyl present in **46** and **47** makes a hydrogen bonding interaction with one of these groups.

Compound **5** displays good in vitro potency against both human and murine 11β-HSD1. We examined tissue inhibition in a mouse ex vivo pharmacodynamic assay (Table 5).[14] In metabolic disease, 11β-HSD1 is dysregulated in adipose tissue and liver, so it is likely that any therapeutic agent to treat type 2 diabetes will have to display inhibition in both tissues. Inhibition of 11β-HSD1 also has an impact on cognition, so we determined whether 11β-HSD1 was inhibited in the CNS. Compound **5** displayed significant inhibition in each of the tissues of interest, but did not inhibit 11β-HSD2 in the kidney. The greatest inhibition was observed in liver and fat, suggesting that optimised compounds of this type may be useful therapeutic agents.

Table 5. Ex vivo pharmacodynamic data for compound **5**

% Inhibition of HSD1 in liver 1 h	% Inhibition of HSD1 in fat 1 h	% Inhibition of HSD1 in brain 1 h	% Inhibition of HSD2 in kidney 1 h
63	54	39	0

Compound was administered at 10 mg/kg i.p.

In summary, we have discovered potent and selective adamantyl amides that display inhibition in liver, adipose and brain following in vivo administration. Optimised analogues may prove to be useful tools to assess the effect of 11β-HSD1 inhibition in vivo.

Acknowledgments

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References and notes

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10. In vitro cellular enzyme inhibition was determined using a scintillation proximity assay (SPA). Human 11β-HSD1 enzyme inhibition was assessed in HEK293 cells stably transfected with the full length human hsd11b1 gene. HEK293 cells were plated in 96-well poly-D-Lys coated flat-bottomed microplates in DMEM containing 1% glutamine, 1% penicillin and streptomycin. Compounds were added to plates such that the final concentration of DMSO was 1%. Tritiated cortisone was added at a final concentration of 20nM and the cells incubated at 37 °C in 5% CO_2, 95% O_2 for 2 h. The assay solutions were transferred to a scintillation microplate and mixed with a solution of anti-mouse YSi SPA beads and anti-cortisol antibody in assay buffer (50mM Tris.HCl, pH 7.0; 300 mM NaCl, 1mM EDTA, 5% glycerol). The plate was incubated for 2 h at room temperature and read on a scintillation counter. The percentage inhibition was determined relative to a non-inhibited control and the median inhibitory concentration (IC_{50}) determined by plotting fractional inhibition against log compound concentration. Data were fitted to the four parameter logistic equation. Murine 11β-HSD1 enzyme inhibition was assessed in CHO cells stably transfected with the full length murine hsd11b1 gene. Enzyme inhibition was determined as described for human 11β-HSD1 following a 4 h incubation of cells, compound and substrate.
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14. Male C57BL/6 mice (25–30 g in weight) were group housed and allowed free access to food and water. Compounds were dissolved in 5% DMSO, 3% ethanol, 4 mM cyclodextrin. Animals ($n = 2$ per group) were dosed intraperitoneally with vehicle or compound at 12-hourly intervals. At 1 h following the third dose mice were euthanized by cervical dislocation. Liver, adipose, kidney and brain samples were removed and frozen until analysis was performed. Inhibition of 11β-HSD1 in each tissue was determined by incubating homogenates with 20 nM tritiated corticosterone, 2mM $NADP^+$ and 0.2% glucose in Krebs buffer, pH 7.4. Dehydrocorticosterone and corticosterone levels were measured by HPLC and the percentage inhibition determined relative to vehicle treated tissue.