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Evaluation of selective inhibitors of 11β-HSD1 for the treatment of hypertension

David R. Bauman [a,†], Alan Whitehead [b,†], Lisa C. Contino [c], Jisong Cui [a], Margarita Garcia-Calvo [d], Xin Gu [b], Nancy Kevin [b], Xiuying Ma [c], Lee-yuh Pai [c], Kashmira Shah [c], Xiaolan Shen [e], Sloan Stribling [c], Hratch J. Zokian [d], Joe Metzger [c], Diane E. Shevell [a,*], Sherman T. Waddell [b,*]

[a] Department of Medicinal Chemistry, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA
[b] Department of Cardiovascular Disease, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA
[c] Department of Drug Metabolism, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA
[d] Department of In Vitro Sciences, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA
[e] Lab Animal Resources, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA

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ABSTRACT

In an effort to understand the origin of blood-pressure lowering effects observed in recent clinical trials with 11β-HSD1 inhibitors, we examined a set of 11β-HSD1 inhibitors in a series of relevant in vitro and in vivo assays. Select 11β-HSD1 inhibitors reduced blood pressure in our preclinical models but most or all of the blood pressure lowering may be mediated by a 11β-HSD1 independent pathway.

Metabolic Syndrome is a complex multifaceted condition characterized by dyslipidemia, hypertension, increased visceral adiposity and loss of glycemic fitness.[1] Simultaneous incremental elevations in these individual risk factors sharply increase the risk of cardiovascular disease and recent mouse studies have suggested a link between intracellular cortisol (in rodents, corticosterone) levels and the development of Metabolic Syndrome-like phenotype.[2] Within key metabolic tissues which express the glucocorticoid receptor (GR), such as liver and adipose, levels of the metabolically active hormone cortisol (which is a ligand for GR) are amplified by the conversion of the inactive precursor cortisone (in rodents, 11-dehydrocorticosterone) to cortisol by type 1 11β-hydroxysteroid dehydrogenase (11β-HSD1). Recently, 11β-HSD1 knock-out (KO) mice fed a high fat diet resisted the development of Metabolic Syndrome whereas overexpression of 11β-HSD1 in mouse adipose tissue led to a Metabolic Syndrome-like phenotype that resulted in hypertension, presumably due to reduced intracellular 11-dehydrocorticosterone and increased intracellular corticosterone levels.[2] In contrast to 11β-HSD1 which increases cortisol levels, the type 2 11β-HSD (11β-HSD2) enzyme converts cortisol to inactive cortisone and can therefore decrease cortisol levels.[3] This is important in tissues that express the mineralocorticoid receptor (MR) such as kidney, in which intracellular cortisol (an MR ligand) concentrations are sharply reduced. This pre-receptor regulation of intracellular cortisol levels by 11β-HSD2 is critical for protecting the MR from excessive cortisol. The importance of 11β-HSD2 is further evidenced by mouse and human genetic variants; loss of 11β-HSD2 function led to a phenotype with apparent mineralocorticoid excess characterized by hypertension, hypokalemia and reduced plasma rennin activity.[4] Taken collectively, 11β-HSD1 represents a target for therapeutic agents that would selectively inhibit 11β-HSD1 and a potential pathway for the treatment of diseases characterized by Metabolic Syndrome.

Two highly potent and selective inhibitors of 11β-HSD1 were discovered in our laboratories.[5] Compounds **1** (MK-0736) and **2** (MK-0916), have been the recent subject of two Phase IIa proof of concept trials in patients with type 2 diabetes and Metabolic Syndrome (Fig. 1).[6] Interestingly, while having only a small effect on endpoints associated with glycemic fitness, **1** and **2** produced a statistically significant decrease in blood pressure in the treatment groups.[7] These early clinical results focused our efforts on

* Corresponding authors. Tel.: +1 732 594 5364 (D.E.S.).
E-mail addresses: diane_shevell@merck.com (D.E. Shevell), tim_waddell@merck.com (S.T. Waddell).
† These authors contributed equally to this work.

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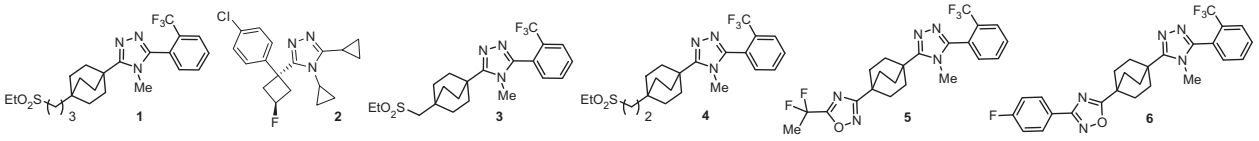
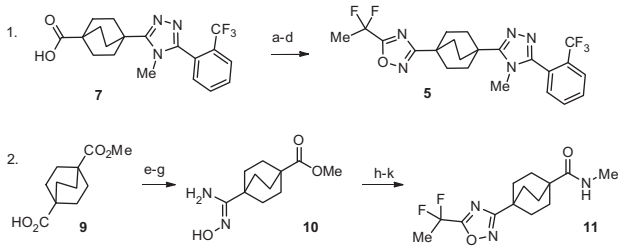


Figure 1. Selected inhibitors of 11β-HSD1 based on blood-pressure lowering effects in SHR model.



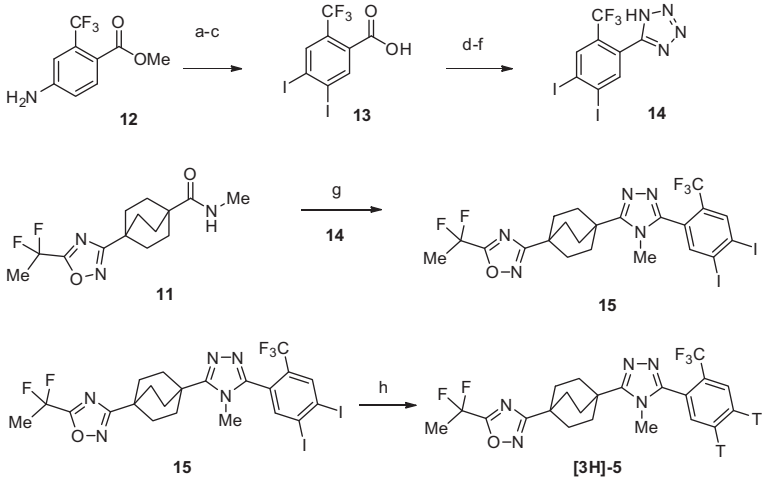
Scheme 1. Reagents and conditions: (a) CDI, $NH_4^+OH^-$, DCM, 96%; (b) trichlorotriazine, DMF, 62%; (c) NH_2OH, EtOH, 100 °C, 98%; (d) $MeCF_2CO_2H$, PyBroB, DIEA, DCM, then reflux, 62%; (e) CDI, $NH_4^+OH^-$ DCM, 86%; (f) trichlorotriazine, DMF, 80%; (g) NH_2OH, EtOH, 90 °C, 98%; (h) $MeCF_2CO_2H$, PyBroB, DIEA, DCM, then reflux, 66%; (i) KOH, MeOH, 98%; (j) $(COCl)_2$, DMF, DCM, then MeNH2, 56%.

investigating blood pressure lowering following 11β-HSD1 inhibition, in order to identify better 11β-HSD1 inhibitors for the treatment of hypertension. In this study, we examined two structural subclasses of 11β-HSD1 inhibitors, one which was very effective at lowering systolic blood pressure (SBP) in the spontaneously hypertensive rat (SHR) model and the other which was only modestly effective at lowering SBP. These compounds had similar in vitro potencies and total exposure levels in SHR plasma. Of particular interest was the question of whether differential tissue exposure (e.g., brain exposure) was responsible for this difference in lowering blood pressure.

In a recent report, the synthesis and in vitro/in vivo data for the class of sulfones exemplified by **1** and **4** were detailed.[8] The preparation of these compounds builds upon the functionally differentiated adamantyl synthon **9**, in a manner similar to the strategy for **5** outlined in Scheme 1.[9] For the synthesis of **5**, beginning with the already installed right-hand 1,2,4-triazole**7**,[10] installation of the 1,2,4-oxadiazole was achieved in an efficient 4-step sequence ending in the coupling of difluoropropionic acid with a transient N-hydroxyamidine. To develop a radio-labeled 11β-HSD1 compound, the ubiquitous aryl group on the right hand side provided a strategically sound position for the introduction of a bis-iodinated aryl group (Scheme 2). To streamline the synthesis of the radio-labeled precursor, the left-hand oxadiazole was first installed starting from **9** using the same 4-step procedure en route to methyl amide **11**.

The requisite iodinated aryl group was prepared in a straightforward process starting from **12** through the regioselective introduction of iodide α to the aniline nitrogen with subsequent manipulation en route to **13**. Conversion of the acid to the reactive *o*-trifluoroaryl tetrazole **14** enabled coupling of the sterically congested chloroimidate intermediate of **11** to proceed, albeit in modest yield, producing bis-iodo **15** as a precursor for radio-labeling.[11] Finally, tritium was introduced by action of Pd/C in the presence of T_2, yielding tracer **[3H]-5** with a specific activity of 40.3 Ci/mmol.

For the discovery program that produced compounds **1**–**6**, routine screening for HSD potency and selectivity was accomplished using a functional SPA assay employing microsomes isolated from stable cell lines expressing human 11β-HSD1 or 11β-HSD2 and measuring the production of radiolabeled cortisol from radiolabeled cortisone. The data set provided by this assay allowed for the identification of compounds that both potently and selectively inhibited 11β-HSD1 as compared to 11β-HSD2, among them **3**–**6** (Table 1). To confirm that these compounds directly interacted with 11β-HSD1 a radiometric direct binding assay was developed using rat liver microsomes with **[3H]-5**. Binding was specific, saturable and consistent with a single molecular site (data not shown). The radiometric binding data indicated that **[3H]-5** potently bound to 11β-HSD1 with nanomolar affinity (K_d = 11 nM) and that the number of liver binding sites (B_{max}) was approximately 55,500 fmol/mg, which is consistent with its high expression level in liver (mRNA and protein) (data not shown). Inhibition constants (K_i) for **3**–**6** were determined using a radiometric competition assay and indicated that these compounds potently bound to 11β-



Scheme 2. Reagents and conditions: (a) Ag_2SO_4, I_2, EtOH, 91%; (b) KI, $NaNO_2$, I_2, CH_3CN, 2M aq HCl, 86%; (c) NaOH, MeOH, 45 °C, 99%; (d) CDI, NH_4OH, DCM, 93%; (e) cyanuric chloride, DMF, 78%; (f) NaN_3, $ZnCl_2$, DMF, 61%; (g) (i) $(ClCO)_2$, DMF, DCM, (ii) Compound **14**, $PhCH_3$, reflux, 21%; (h) Pd/C, $Pd/CaCO_3$, T_2.

Table 1
Biochemical properties of the 11β-HSD1 inhibitors

Compound	Human 11β-HSD2; IC_{50} (nM)	Human 11β-HSD1; IC_{50} (nM)	Human IC_{50} (11β-HSD2/ 11β-HSD1)	Rat 11β-HSD1; IC_{50} (nM)	Rat K_i (nM)
3	>4000	31.2	>128	9.1	13.1
4	>4000	7.5	>530	6.0	3.9
5	3100	30.4	102	4.5	7.0
6	2200	3.0	732	3.0	1.3

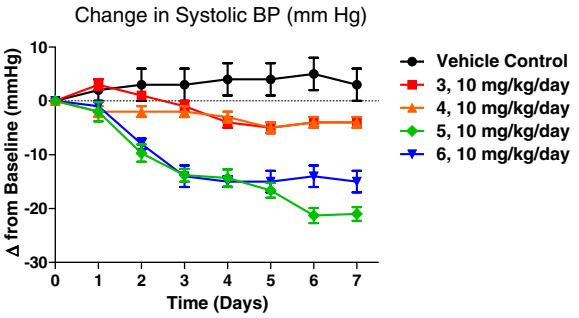


Figure 2. SHR changes in systolic blood pressure treated with 11β-HSD1 inhibitors for 7-days.

HSD1 with values similar to the functional enzyme inhibition (SOLSPA) data (Table 1).

Selectively potent inhibitors of 11β-HSD1 were tested in the SHR model to determine in vivo hemodynamic profiles. SHR were telemeterized with abdominal aorta catheters, dosed daily with 10 mg/kg compound and hemodynamic parameters recorded (heart rate, systolic and diastolic blood pressure). After several days of dosing, **3**–**6** significantly lowered both systolic and diastolic BP in SHRs compared to vehicle treated animals (Fig. 2).

These compounds did lower heart rate in SHR and some of these compounds did increase body weight (data not shown). Closer analysis of the SHR data revealed that compounds could be differentiated based on BP efficacy into two groups: low and high effect compounds. On average, the low effect compounds (represented by **3** and **4**) typically lowered SBP in the range of 3-8 mmHg in SHR, while the high effect compounds (represented by **5** and **6**) lowered SBP by more than 15 mmHg in SHR. Although all of these compounds had similar in vitro biochemical properties, they exhibited very distinct in vivo efficacies in SHR, suggesting that some other biophysical property was different between the two groups (low vs high effect compounds), yet similar within each group (high vs high effect compounds). Several hypotheses were tested to investigate this in vivo BP efficacy difference.

The first hypothesis tested was that PK differences were the underlining cause for the BP efficacy difference. After compound administration, plasma drug exposures were determined at peak (~4 h post dose) and trough (~24 h post dose) for **3**–**6**. The resulting data indicated similar total plasma peak concentrations between the low and high effect compounds. However, trough level differences were identified whereby high effect compounds had slightly to significantly higher total concentrations at trough time points compared to the low effect compounds (Table 2).

One explanation for the difference in BP efficacy was that sustained exposure is needed to maintain lower BP. However, the high effect compound **6** had the lowest free drug plasma concentration, suggesting that plasma drug exposure (total or free drug) was a poor indicator of BP efficacy. A second hypothesis tested was that brain exposure might be necessary for a substantial antihypertensive effect. Consequently, we set out to determine if there was a difference in brain exposure between the high and low effect compounds. Susceptibility to PGP was measured, and as indicated in Table 2, the high effect compounds were not PGP substrates (BA/AB ratio = 1.5 for **5** and 0.7 for **6**), whereas the low effect compound tested was a PGP substrate (BA/AB ratio = 10.2 for **4**), consistent with the idea that the high effect compounds might have greater brain exposures. To verify that brain exposure differences existed between low and high effect compounds, rats were dosed with 10 mg/kg compound, sacrificed at the indicated time, the brains isolated, and a detailed PK study was performed for **3**–**6** (Table 2). The resulting data gave brain to plasma ratios consistent with the idea that the high effect compounds were not PGP substrates (Brain/Plasma ratio >1 for **5** and **6**) and that the low effect compounds were PGP substrates (Brain/Plasma ratio <1 for **3** and **4**). Comparing total brain drug exposure between the two classes appeared to support the idea that brain coverage was important for the observed in vivo efficacy difference; however, more detailed PK analyses indicated similar free drug levels in brain for both low (**3**, 1.3 μM; **4**, 0.5 μM) and high (**5**, 3 μM; **6**, 0.08 μM) effect compounds. Since unbound drug levels drive receptor occupancy, this suggested that central exposure was not the main driver in the observed BP efficacy difference.

To directly explore the origin of the observed BP lowering with our 11β-HSD1 inhibitors, **5** was tested in telemeterized 11β-HSD1 KO mice. Interestingly, in the complete 11β-HSD1 KO a reduction in baseline hemodynamic parameters was observed for SBP (KO 120 ± 1.1 mmHg vs WT 127 ± 1.4 mmHg), DBP (KO 91 ± 1.0 mmHg vs WT 98 ± 0.9 mmHg) and heart rate (KO 585 ± 4 bpm vs WT 595 ± 6 bpm), although body weight (KO 30 ± 0.6 g vs WT 29 ± 0.4 g) and activity (KO 8 ± 1.1 counts/min vs WT 7 ± 0.4 counts/min) were similar between the two genotypes. As the KO mice and WT controls are not littermates, this data does not prove that loss of 11β-HSD1 lowers BP, but suggests a role for 11β-HSD1 in blood pressure regulation.

Subsequently, 11β-HSD1 KO and WT animals were dosed with **5** to determine if the additional BP lowering effect observed in SHR for high effect compounds was due to an off-target activity. As seen in Figure 3, **5** had similar effects on SBP lowering in both 11β-HSD1 WT (−8 mmHg) and KO (−5.8 mmHg) mice with similar exposure (WT = 12.1 μM and KO = 13.1 μM for **5**), indicating that most or all of the BP lowering ability of high effect compounds was due to an

Table 2
Plasma and brain exposure for the 11β-HSD1 inhibitors

Compound	Time (h)	Plasma total (μM)	Plasma free (μM)	Brain total (μM)	Brain free (μM)	CSF (μM)	PGP ratio
3	4	27.7 ± 5.4	8.0 ± 2.5	3.1 ± 0.6	0.5 ± 0.1	2.1 ± 0.5	10.2
	24	10.2 ± 4.2	2.1 ± 1.1	0.8 ± 0.2	0.2 ± 0.1	0.7 ± 0.4	10.2
4	4	24.2 ± 2.6	9.5 ± 1.2	5.0 ± 0.8	1.3 ± 0.2	5.1 ± 1.2	N/D
	24	6.0 ± 4.2	1.9 ± 1.2	0.7 ± 0.4	0.2 ± 0.2	0.7 ± 0.5	N/D
5	4	25.0 ± 1.1	2.5 ± 0.6	60.5 ± 6.9	3.0 ± 0.2	4.4 ± 1.0	1.45
	24	25.4 ± 1.6	2.4 ± 0.5	55.4 ± 5.9	2.4 ± 0.7	3.4 ± 2.2	1.45
6	4	13.9 ± 1.7	0.07 ± 0.02	44.4 ± 11.7	0.08 ± 0.03	0.2 ± 0.2	0.7
	24	12.1 ± 2.3	0.05 ± 0.01	39.1 ± 3.8	0.06 ± 0.01	0.2 ± 0.1	0.7

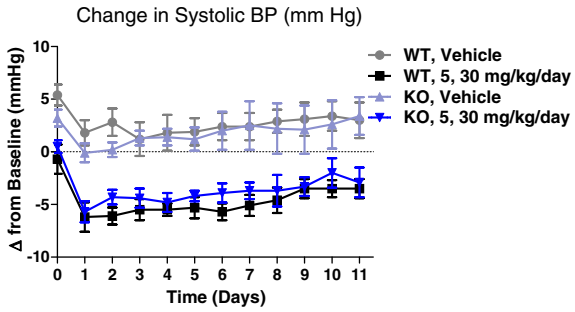


Figure 3. 11β-HSD1 KO and WT mice changes in systolic blood pressure treated with compound **5** for 11-days.

unknown off-target activity. In addition the decrease in HR and increase in BW observed with **5** also appears to be off-target.

In an effort to better understand BP lowering observed in the clinic after treatment with 11β-HSD1 inhibitor drugs, we studied two sets of potent, selective adamantyl triazole inhibitors of 11β-HSD1, which differed in their ability to reduce BP in the SHR model. Since the 'high BP effect' compounds were not substrates for PGP whereas the 'low BP effect' compounds were PGP substrates, a leading hypothesis at the beginning of this study was that central (brain, CNS) coverage of 11β-HSD1 might be necessary for the antihypertensive effect. Although total brain levels were higher for the 'high BP effect' compounds, free-drug brain levels measured for both classes were similar, leading to the conclusion that it is unlikely that central coverage is the main driver of the antihypertensive effect.

To further probe the origin of the apparent antihypertensive effect of HSD inhibition, we produced telemetrized 11β-HSD1 KO mice. Significant reductions in 11β-HSD1 KO mice baseline hemodynamic profiles suggest an 11β-HSD1 dependent pathway in BP homeostasis. However, as **5** lowered BP equally in both 11β-HSD1 KO and WT mice, the BP reduction difference observed in SHR between high and low effect compounds is most likely due to an unknown off-target activity. In summary, our comparative investigation of both high and low BP effect 11β-HSD1 inhibitors suggests that BP lowering for this series of compounds operates through an unknown off-target mechanism.

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