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Letters

Arylsulfonamidothiazoles as a New Class of Potential Antidiabetic Drugs. Discovery of Potent and Selective Inhibitors of the 11 β -Hydroxysteroid Dehydrogenase Type 1

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Abstract: Novel antidiabetic arylsulfonamidothiazoles are presented that exert action through selective inhibition of the 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) enzyme, thereby attenuating hepatic gluconeogenesis. The diethylamide derivative **2a** was shown to potently inhibit human 11 β -HSD1 (IC₅₀ = 52 nM), whereas the *N*-methylpiperazinamide analogue **2b** only inhibited murine 11 β -HSD1 (IC₅₀ = 96 nM). Both compounds showed >200-fold selectivity over human and murine 11 β -HSD2. **2b** was subsequently shown to reduce glucose levels in diabetic KKA^y mice, substantiating the 11 β -HSD1 enzyme as a target for the treatment of type 2 diabetes.

Excessive levels of glucocorticoids can cause metabolic complications. Glucocorticoid action is dependent on circulating hormone levels and local activation or re-

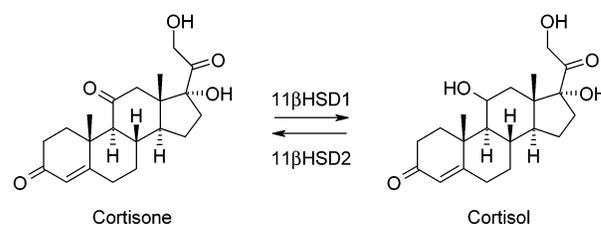


Figure 1. Interconversion of cortisone and cortisol by 11 β -HSD types 1 and 2 enzymes.

generation by key enzymes. Cortisol and the inactive counterpart cortisone may be interconverted by 11 β -hydroxysteroid dehydrogenases (in rodents corticosterone and 11-dehydrocorticosterone) (Figure 1).¹ The type 1 isoform of 11 β -hydroxysteroid dehydrogenase (11 β -HSD1) is a reductase and highly expressed in the liver. Gluconeogenesis in this organ is reduced when the 11 β -HSD1 gene is knocked out, resulting in lower fasting blood glucose levels.² Recent data suggest that improved insulin release might contribute to resistance to hyperglycemia in 11 β -HSD1 $-/-$ mice.³

The second isoform, 11 β -HSD2, inactivates cortisol by converting it to cortisone. Disruption or mutations in the 11 β -HSD2 gene result in sodium retention, hypokalemia, and hypertension because of inappropriate glucocorticoid occupation of the mineralocorticoid receptor in the kidney.⁴

Various compounds have previously been shown to inhibit 11 β -HSD1, but none of these were reported to be 11 β -HSD1 selective, potent, and druglike.^{5,6} For example, the endogenous steroid chenodeoxycholic acid is selective but not potent (*K_i* in the micromolar range) and might not be considered druglike.⁵ Glycyrrhetic acid and the hemisuccinyl derivative carbenoxolone, the only known potent inhibitors to date, display poor selectivity between the two isoforms of 11 β -HSD.^{5,6} Carbenoxolone has earlier been demonstrated to improve insulin sensitivity and to decrease glucose production in healthy human volunteers.⁷ In that study, the 11 β -HSD2 inhibitory activity of carbenoxolone was a limiting factor because it induces renal mineralocorticoid excess at higher doses. Thus, while inhibition of

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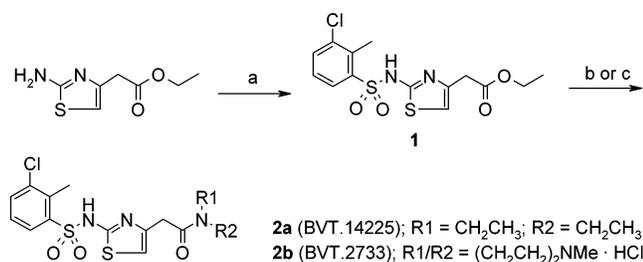
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Scheme 1. Synthesis of Arylsulfonamidothiazoles^a

^a (a) 3-Chloro-2-methylphenylsulfonyl chloride, pyridine; (b) AlCl₃, *N,N*-diethylamine, DCM; (c) (i) KOH, EtOH, (ii) *N*-methylpiperazine, EDCl, HOBT, TEA, DCM.

the 11 β -HSD1 enzyme could provide a very attractive and novel treatment of type 2 diabetes, it is obvious that an inhibitor against 11 β -HSD1 must not affect 11 β -HSD2. This communication describes the discovery of the first potent and selective druglike inhibitors of 11 β -HSD1.

A high-throughput screen of our compound collection followed by a lead optimization process provided us with arylsulfonamidothiazole **2a** and **2b** as examples of this structural class (Scheme 1). Compounds of interest were prepared as follows. Ethyl (2-aminothiazol-4-yl)acetate was sulfonylated with 3-chloro-2-methylbenzenesulfonyl chloride in pyridine in 79% yield. The resulting ester intermediate can directly be treated with *N,N*-diethylamine in the presence of aluminum chloride in dichloromethane, giving compound **2a** in 68% yield. Hydrolysis of the ethyl ester **1** in ethanol using potassium hydroxide gave the carboxylic acid in a high yield. Treatment of the intermediate acid with *N*-methylpiperazine using 1-hydroxybenzotriazole and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride as amide coupling reagents afforded compound **2b** as the hydrochloride salt in a moderate 48% yield.

To evaluate the ability of the compounds to inhibit human or mouse 11 β -HSD1, they were assayed in a scintillation proximity assay (SPA). Recombinant human and murine 11 β -HSD1 were produced in *Pichia pastoris*^{6,8} and treated with a substrate/cofactor mixture of [³H]-cortisone/NADPH and different concentrations of inhibitor. The conversion of cortisone to cortisol was stopped by addition of glycyrrhetic acid. The labeled cortisol product was captured by mouse monoclonal anticortisol antibodies (6D6.7) and was subsequently bound to SPA beads coated with antimouse antibodies. The amount of [³H]-cortisol bound to the beads was determined in a scintillation counter.

Assessment of human 11 β -HSD2 activity was based on the conversion of [³H]-cortisol to the tritium-labeled product (cortisone) in the presence of inhibitor. The enzymatic reaction was performed with a surplus of the cofactor NAD⁺ and stopped with perchloric acid. Substrate and product were separated by HPLC and monitored using a flow scintillation counter. Enzyme activity was quantified as the percentage area of the product peak compared to the total area.

Since the mouse and human 11 β -HSD1 enzymes display only 79% amino acid identity, we were not surprised that the compounds did not inhibit 11 β -HSD1 from both species to a similar degree (Table 1). Apparently, the arylsulfonamidothiazole moiety can be accommodated in both 11 β -HSD1 orthologues while the

Table 1. In Vitro Inhibitory Activities for the 11 β -HSD1 and 11 β -HSD2 Enzymes

compound	IC ₅₀ (nM) \pm SEM ^a		
	mouse 11 β -HSD1	human 11 β -HSD1	human 11 β -HSD2
carbenoxolone	108 \pm 6	330 \pm 16	83 \pm 5 ^b
2a	284 \pm 24	52 \pm 3	> 10000
2b	96 \pm 14	3341 \pm 412	> 10000

^a IC₅₀ values (\pm SEM) for inhibition of the 11 β -HSD1 catalyzed formation of [³H]-cortisol from cortisone or vice versa for 11 β -HSD2. Values were obtained with 11 inhibitor concentrations from three to seven independent determinations, each performed in triplicate. ^b Value was obtained with 11 inhibitor concentrations from two independent determinations, each performed in triplicate.

right-hand side of the molecule determines the extent of inhibition of either murine or human 11 β -HSD1. The more flexible diethylamide group of **2a** is well accommodated in the human enzyme, whereas the *N*-methylpiperazinamide group of **2b** is not at all tolerated because of steric reasons or maybe its basic character. In contrast, this group is well accepted in the mouse 11 β -HSD1 enzyme. Inhibition of both enzymes was evaluated with cortisone as the substrate, which is the natural substrate for the human enzyme.

Importantly, the arylsulfonamidothiazole derivatives exhibited no appreciable inhibitory activity against human 11 β -HSD2, and **2b** showed no inhibition of the mouse 11 β -HSD2 enzyme at 0.2 mM concentration (data not shown). Thus, **2a** and **2b** are more than 200-fold selective for the human and mouse 11 β -HSD1 enzymes, respectively. Carbenoxolone was less effective than **2a** in inhibiting human 11 β -HSD1 and was shown to potentially inhibit the human 11 β -HSD2 enzyme with an IC₅₀ value of 83 nM. A calculation using the published *K_m* value of 25.5 nM yields a *K_i* of 11 nM, well in line with earlier reported *K_i* values.^{5,9} Unlike the arylsulfonamidothiazole derivatives, carbenoxolone displays negligible ability to discriminate between the mouse and human 11 β -HSD1 enzymes.

The favorable pharmacokinetic and in vitro selectivity profile of compound **2b** allowed us to assess the proposed importance of the 11 β -HSD1 enzyme for the treatment of diabetes in the hyperglycemic KKA^y mouse model. Pharmacokinetic experiments with compound **2b** at doses of 10–100 mg/kg yielded a serum terminal half-life in the range 2.5–3.5 h and an oral bioavailability of approximately 21% in the mouse.

Indeed, we found that compound **2b** (BVT.2733; 25, 50, 100 mg/kg po) administered twice daily significantly lowered blood glucose levels in a dose-dependent manner in KKA^y mice (*n* = 12–13), as measured on days 3–11 (Figure 2). The maximal reduction in glucose was 53% of the control after 11 days of treatment at the highest dose. Serum compound levels 12 h after the last administration were 0.06 \pm 0.02, 0.13 \pm 0.02, and 0.19 \pm 0.04 μ M, respectively. The plasma-free fraction of **2b** in serum amounted to 5%, indicating that at the highest doses used, enough unbound compound is available to

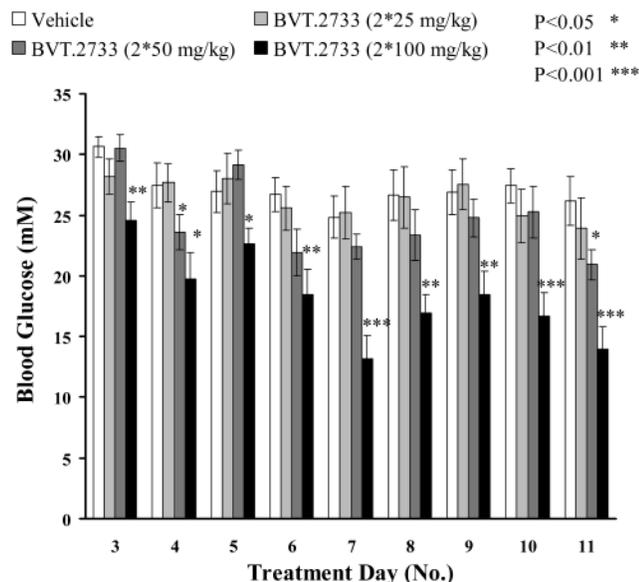


Figure 2. Glucose-lowering effect of **2b** in KKA^y mice.

exert the pharmacological effect observed. Compound **2b** did not alter liver or heart marker enzymes (AST, ALT, ALP), suggesting that liver impairment can be excluded in the present experiment. From evidence generated in a different experiment, we inferred that reduced hepatic glucose output contributes to the glucose-lowering effect obtained here. A 1-week treatment of KKA^y mice with **2b** (167 mg/kg/day) using osmotic minipumps lowered mRNA levels encoding for two key enzymes in hepatic glucose production: phosphoenolpyruvate carboxykinase (75% of control; $P < 0.05$) and glucose-6-phosphatase (55% of control; $P = 0.089$).¹⁰

Our data support the literature finding that 11 β -HSD1 is a key mediator of gluconeogenesis.² The results presented in this paper strengthen 11 β -HSD1 inhibition as an interesting approach for type 2 diabetes treatment, and pharmacological tools have been developed to explore the potential of this concept further. Development of compound **2a**, a selective inhibitor of the human 11 β -HSD1 enzyme, is the first step toward the clinical evaluation of this novel compound class aimed to benefit type 2 diabetic patients without the risk of hypertension.

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Supporting Information Available: Experimental section containing preparation of **2a** and **2b** and description of

the in vitro inhibition assays and in vivo pharmacology. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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