**Inhibition of 11-hydroxysteroid dehydrogenase type 2 as a potential treatment for hyponatremia**

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XXX. Patients with loss-of-function mutations in *11β-HSD2* (11β-hydroxysteroid dehydrogenase type 2) are characterized by sodium retention. The direct inhibition of this enzyme could therefore represent a potential therapy for chronic hyponatremia, a condition that is prevalent among elderly patients. To identify agents with the ability to bind and modulate 11β-HSD2 specifically, we performed a virtual screen of several compound libraries using a newly developed algorithm for detecting compounds with the required physicochemical and structural characteristics. The top X candidate compounds identified by the virtual screen were evaluated for their ability to interact with recombinant human 11β-HSD2. One compound, DE2300c5, strongly bound to 11β-HSD2 without affecting either 11β-HSD1 or 17β-HSD2. Binding was confirmed *in vitro* using HEK293 cells, and administration of DE2300c5 increased intracellular sodium levels in renal cortical cells. miR-401 was unchanged by DE2300c5 administration. The findings of the present study suggest that DE2300c5 may be a potential treatment for hyponatremia, which is a significant health burden, and other electrolyte imbalances.

**INTRODUCTION**

XXX. The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) catalyzes the conversion of the biologically active steroid hormone cortisol to its inactive form, cortisone (Fig. 1). This reaction is co-factor dependent and relies on NAD+2. Cortisol binds to the MR as tightly as its other natural ligand, aldosterone. In vivo, 11β-HSD2 is co-localized in tissues where expression of the MR is high; the main role the enzyme in this setting is to regulate the local concentration of cortisol and thereby prevent excessive binding to the MR. Activation of the MR results in re-absorption of sodium ions, excretion of potassium ions and an associated increase in blood pressure. The MR is a member of the nuclear receptor family, and it transcriptionally regulates the alpha subunit of the epithelial Na(+) channel as well as other ion transport machinery.

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[Other text deleted]**Dose-Response Curves**

The 11β-HSD2 inhibition assays were performed at room temperature (25°C) in 35 mM Tris buffer, pH 8.0, containing 20 mM NaCl. The total reaction volume in each well of the plate was 100 μl and contained the following: 30 μl of each inhibitor (see Supplementary Information), 30 μl of 10 mM NADH, 30 μl of 4 mM cortisol and 60 μl of 160 μg/ml 11β-hydroxysteroid dehydrogenase-2. The reaction mixture was incubated at 25°C and then the substrate was added. The product was transferred into the wells before fluorescence was measured using a fluorometer. Each reaction was performed in triplicate using the inhibitors at different concentrations (see Supplementary Information). A master mix was used to initiate the reaction, and blanks for all inhibitors were included as controls. The pH of each reaction was determined.

**Crystallographic analysis**

Crystallization trials were performed with bound DE2300c5 to investigate the mechanism of ligand binding. Human recombinant 11β-HSD2 was overexpressed in the BL21 strain of *E. coli*, and the purified extract was determined to be 98% pure using SDS-PAGE gels and a densitometric analysis. The Bradford protein assay was used to determine the concentration of the purified extract. The enzyme was then concentrated in 500 μM NADH buffer containing 1% glycerol, 1 mM EDTA, 50 mM Tris, pH 8, and 100 mM NaCl. The enzyme was then assayed for activity. Finally, the sample was centrifuged at 2200 rpm for 5 to 10 minutes at 5°C to remove dust, precipitated protein, etc. Human recombinant 11β-hydroxysteroid dehydrogenase-2 was crystallized at a final concentration of 32 mg/ml in pH 7.8 crystal buffer (1% glycerol, 30 mM Tris, 45 mM NaCl, 5 mM EDTA).

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**Figure 3.** The inhibition of 11β-HSD2 from each potential inhibitor in transfected cells. DE2300c5 is compound 8. The experiments were performed using HEK293 cells.

**Figure 4.** The relationship between counts per minute (as detected by scintillation counting) and inhibitor concentration for 11β-HSD2-transfected HEK293 cells. A dose-response plot is shown for each candidate 11β-HSD2 inhibitor. The chemical structure and the IC50 are also shown. The maximum line corresponds to substrate binding in the absence of a competitor. DE2300c5 is compound 8. Error bars = S.E., N = 2.

**Table 1.** A summary of the results from the scintillation proximity assay (SPA) for six selected compounds. Please see the Supplementary Information for further details.

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| **Compound number**  | **IC50 cell SPA**  | **Calculated K i** **of SPA (cell)** | **Calculated Ki of SPA (recombinant)** |
| 3  | 10.4 ± 1.1 μM  | 1.2 μM  | N/A  |
| 5  | 1.8 ± 4.04 μM  | 2.5 μM  | N/A |
| DE2300c5  | 1.82 ± 0.03 nM  | 15 nM  | 1.7 nM ± 0.3  |
| 9 | 10.11 ± 8.1 μM | 2.8 μM | N/A |
| 10  | 9.61 ± 12.4 μM  | 5.7 μM  | N/A |
| 21  | 6.73 ± 2.06 μM  | 2.2 μM  | N/A  |

**Table 2.** Summary information relating to the top compounds inhibiting 11β-HSD2 and the query molecules.

[Table and other text deleted]